

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
21 December 2000 (21.12.2000)

PCT

(10) International Publication Number
WO 00/77255 A1

- (51) International Patent Classification: C12Q 1/68, C12N 15/09, 7/00, 5/00, C07H 21/04, C07K 17/00, 1/00, 14/00, 16/00, A61K 39/29
- (74) Agents: HOOVER, Kenley, K. et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).
- (21) International Application Number: PCT/US00/14926
- (81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 1 June 2000 (01.06.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/138,628 11 June 1999 (11.06.1999) US
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): ROSEN, Craig, A. [US/US]; 22400 Rolling Hill Road, Laytonville, MD 20882 (US). RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Laytonville, MD 20882 (US). KOMATSOUKIS, George, A. [US/US]; 9518 Garwood Street, Silver Spring, MD 20901 (US).
- Published:**
- With international search report.
 - Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 00/77255 A1

(54) Title: 49 HUMAN SECRETED PROTEINS

(57) Abstract: The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

49 Human Secreted Proteins

Field of the Invention

This invention relates to newly identified polynucleotides and the polypeptides encoded by these polynucleotides, uses of such polynucleotides and polypeptides, and their production.

Background of the Invention

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly,

proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

Summary of the Invention

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

Detailed Description

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as

disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain
5 the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the
10 nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the
15 polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As
20 shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

25 A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to

sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x

- 5 Denhardt's solution, 10% dextran sulfate, and 20 μ g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions.

- Changes in the stringency of hybridization and signal detection are primarily
10 accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4 ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 μ g/ml
15 salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

- Note that variations in the above conditions may be accomplished through the
20 inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above,
25 due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more

- detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in
- 5 a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation,
- 10 ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation,
- 15 gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES,
- 20 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)
- "SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y"
- 25 refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Many proteins (and translated DNA sequences) contain regions where the amino acid composition is highly biased toward a small subset of the available residues. For example, membrane spanning domains and signal peptides (which are also membrane spanning) typically contain long stretches where Leucine (L), Valine (V), Alanine (A), and Isoleucine (I) predominate. Poly-Adenosine tracts (polyA) at the end of cDNAs appear in forward translations as poly-Lysine (poly-K) and poly-Phenylalanine (poly-F) when the reverse complement is translated. These regions are often referred to as "low complexity" regions.

Such regions can cause database similarity search programs such as BLAST to find high-scoring sequence matches that do not imply true homology. The problem is exacerbated by the fact that most weight matrices (used to score the alignments generated by BLAST) give a match between any of a group of hydrophobic amino acids (L,V and I) that are commonly found in certain low complexity regions almost as high a score as for exact matches.

In order to compensate for this, BLASTX.2 (version 2.0a5MP-WashU) employs two filters ("seg" and "xnu") which "mask" the low complexity regions in a

particular sequence. These filters parse the sequence for such regions, and create a new sequence in which the amino acids in the low complexity region have been replaced with the character "X". This is then used as the input sequence (sometimes referred to herein as "Query" and/or "Q") to the BLASTX program. While this regime helps to ensure that high-scoring matches represent true homology, there is a negative consequence in that the BLASTX program uses the query sequence that has been masked by the filters to draw alignments.

Thus, a stretch of "X"'s in an alignment shown in the following application does not necessarily indicate that either the underlying DNA sequence or the translated protein sequence is unknown or uncertain. Nor is the presence of such stretches meant to indicate that the sequence is identical or not identical to the sequence disclosed in the alignment of the present invention. Such stretches may simply indicate that the BLASTX program masked amino acids in that region due to the detection of a low complexity region, as defined above. In all cases, the reference sequence(s) (sometimes referred to herein as "Subject", "Sbjct", and/or "S") indicated in the specification, sequence table (Table 1), and/or the deposited clone is (are) the definitive embodiment(s) of the present invention, and should not be construed as limiting the present invention to the partial sequence shown in an alignment, unless specifically noted otherwise herein.

20

Polynucleotides and Polypeptides of the Invention

FEATURES OF PROTEIN ENCODED BY GENE NO: 1

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting

example, the sequence accessible through the following database accession no. gil3002527 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "neuronal thread protein AD7c-NTP [Homo sapiens]". A partial alignment demonstrating the observed

5 homology is shown immediately below.

```

>gi|3002527 (AF010144) neuronal thread protein AD7c-NTP [Homo sapiens]
>sp|O60448|O60448 NEURONAL THREAD PROTEIN AD7C-NTP.
Length = 375

10      Minus Strand HSPs:

      Score = 152 (53.5 bits), Expect = 1.3e-11, Sum P(2) = 1.3e-11
      Identities = 38/73 (52%), Positives = 40/73 (54%), Frame = -1

15      Q: 1824 LRCSFALVAQAGVQWCDLGSLQXXXXXXXXXXXXXRLLSGWDYRHPFPCLTNFLYF*KRWG 1645
           LR S   V QAGVQW +IGSLQ                               LLS WDYR PP L NF F   G
      S: 202  LRQSSINSVTQAGVQWRNLGSLQLPLPGFKLFSCPSLLSSWDYRRPPR-LANFFVFLVEMG 260

20      Q: 1644 FTMRLARLLLNS*P 1606
           FTM ARL+L S P
      S: 261 FTMFARLILISGP 273

```

The segment of gil3002527 that is shown as "S" above is set out in the

25 sequence listing as SEQ ID NO. 109. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

30 Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 110 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following

35 tissues/cDNA libraries: Chromosome 7 Placental cDNA Library; Chromosome 7

- Fetal Brain cDNA Library; Human brain cDNA and to a lesser extent in Human Tonsil, Lib 3; Healing groin wound, 7.5 hours post incision; Jurkat T-Cell, S phase; Soares_testis_NHT; Human Activated T-Cells, re-excision; Chromosome 7 HeLa cDNA Library; NCL_CGAP_GCB1; NCL_CGAP_Lym3; NCL_CGAP_Phe1; Human
- 5 Ovarian Cancer Reexcision; human tonsils; Neutrophils IL-1 and LPS induced; H. Frontal cortex,epileptic,re-excision; Human 8 Week Whole Embryo and T cell helper II.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
- 10 related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
- 15 general formula of a-b, where a is any integer between 1 to 1873 of SEQ ID NO:11, b is an integer of 15 to 1887, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

20 FEATURES OF PROTEIN ENCODED BY GENE NO: 2

- The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi4102711 (all information available through the recited accession number is
- 25 incorporated herein by reference) which is described therein as "EH-binding protein

[Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >gi|4102711 (AF015043) EH-binding protein [Homo sapiens]
      >sp|G4102711|G4102711 EH-BINDING PROTEIN (FRAGMENT).
          Length = 479

      Plus Strand HSPs:

10      Score = 195 (68.6 bits), Expect = 2.4e-11, P = 2.4e-11
          Identities = 38/38 (100%), Positives = 38/38 (100%), Frame = +3

      Q:   243 NPITKRWKHLTGTLILVNSLDVLRAAAFSPADQDDFVI 356
          NPITKRWKHLTGTLILVNSLDVLRAAAFSPADQDDFVI
15      S:   442 NPITKRWKHLTGTLILVNSLDVLRAAAFSPADQDDFVI 479
  
```

The segment of gi|4102711 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 111. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities.

20 Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 112 which
 25 corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Endometrial Tumor and to a lesser extent in Soares_fetal_heart_NbHH19W; Soares melanocyte 2NbHM; Soares fetal liver spleen
 30 1NFLS; Soares_multiple_sclerosis_2NbHMSP; Human Gall Bladder; Human Fetal Kidney, Reexcision; Soares infant brain 1NIB; Soares ovary tumor NbHOT; Stratagene endothelial cell 937223; H. Kidney Medulla, re-excision; Stratagene colon (#937204); NCI_CGAP_Kid3; Colon Tumor II;

- Soares_multiple_sclerosis_2NbHMSP; Kidney Pyramids; Soares_NFL_T_GBC_S1; Soares_fetal_heart_NbHH19W; Soares_NSF_F8_9W_OT_PA_P_S1; normalized infant brain cDNA; Saos2 Cells, Vitamin D3 Treated; Human (HCC) cell line liver (mouse) metastasis, remake; Human OB HOS treated (10 nM E2) fraction I; Human
- 5 Aortic Endothelium; Human Cardiomyopathy, subtracted; H. Epididymus, caput & corpus; Aorta endothelial cells + TNF-a; Human endometrial stromal cells; Soares_senescent_fibroblasts_NbHSF; Human Pancreas Tumor; Stromal cell TF274; Ulcerative Colitis; Human Adrenal Gland Tumor; Human Liver, normal; Smooth muscle, serum induced, re-exc; Colon Tumor; NCI_CGAP_Lu5;
- 10 Soares_multiple_sclerosis_2NbHMSP; Human Synovial Sarcoma; Human Testes, Reexcision; Human Fetal Heart; Human Adult Pulmonary, re-excision; Endothelial cells-control; Smooth muscle, control; Human 8 Week Whole Embryo; Nine Week Old Early Stage Human and Primary Dendritic Cells, lib 1.

- Preferred polypeptides of the present invention comprise immunogenic
- 15 epitopes shown in SEQ ID NO: 61 as residues: Met-1 to Trp-9. Polynucleotides encoding said polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:12 and may have been publicly available prior to conception of
- 20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2271 of SEQ ID NO:12, b
- 25 is an integer of 15 to 2285, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 3

- 5 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Endometrial Tumor; Soares_multiple_sclerosis_2NbHMSP and to a lesser extent in Soares_fetal_liver_spleen_INFLS_S1; Soares_NFL_T_GBC_S1.

- Many polynucleotide sequences, such as EST sequences, are publicly
10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:13 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
15 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1791 of SEQ ID NO:13, b is an integer of 15 to 1805, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 4

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares_pregnant_uterus_NbHPU and to a lesser extent in Human Endometrial Tumor; Keratinocyte; Soares infant brain INIB; Human
25 Substantia Nigra; Human Amygdala; T Cell helper I; Human 8 Week Whole Embryo; Soares fetal liver spleen INFLS; Supt Cells, cyclohexamide treated; Human Ovary;

- Human Fetal Brain; Soares_NhHMPu_S1; Human Fetal Heart; Human Osteoclastoma; Hodgkin's Lymphoma II; Human Fetal Brain, normalized c5-11-26; Osteoclastoma-normalized A; Osteoclastoma-normalized B; Soares_total_fetus_Nb2HF8_9w; Human osteoarthritic,fraction II; Human Umbilical
- 5 Vein Endothelial cells, frac B, re-excision; Morton Fetal Cochlea; Soares_senescent_fibroblasts_NbHSF; CD40 activated monocyte dendritic cells; Aorta endothelial cells + TNF-a; Soares retina N2b4HR; Soares_pregnant_uterus_NbHPU; human corpus colosum; Human Osteoclastoma, re-excision; Synovial Fibroblasts (III/TNF), subt; wilm's tumor; Human Chronic
- 10 Synovitis; TF-1 Cell Line GM-CSF Treated; Stromal cell TF274; Stratagene endothelial cell 937223; Stratagene ovarian cancer (#937219); Stratagene NT2 neuronal precursor 937230; Human Adipose; Soares_fetal_heart_NbHH19W; Soares_fetal_liver_spleen_INFLS_S1; Hemangiopericytoma; Human Adrenal Gland Tumor; Rejected Kidney, lib 4; CHME Cell Line,treated 5 hrs; Human Liver, normal;
- 15 Human promyelocyte; Soares_testis_NHT; Soares_NFL_T_GBC_S1; Soares_pregnant_uterus_NbHPU; Soares_fetal_liver_spleen_INFLS_S1; Colon Carcinoma; Soares melanocyte 2NbHM; Soares_multiple_sclerosis_2NbHMSP; Primary Dendritic cells,frac 2; Bone marrow; Endothelial-induced; Activated T-Cell (12hs)/Thiouridine labelledEco; Monocyte activated; Human Bone Marrow, treated;
- 20 Spleen, Chronic lymphocytic leukemia; Human Testes; Bone Marrow Cell Line (RS4,11); NCI_CGAP_GC4; NCI_CGAP_Lu5; NCI_CGAP_Ov2; NCI_CGAP_GCB1; Nine Week Old Early Stage Human; Soares placenta Nb2HP and Primary Dendritic Cells, lib 1.

- Many polynucleotide sequences, such as EST sequences, are publicly
- 25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

5 general formula of a-b, where a is any integer between 1 to 1519 of SEQ ID NO:14, b is an integer of 15 to 1533, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

10 **FEATURES OF PROTEIN ENCODED BY GENE NO: 5**

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares melanocyte 2NbHM; NCI_CGAP_GCB1; Soares_fetal_liver_spleen_1NFLS_S1 and to a lesser extent in Synovial IL-1/TNF stimulated; Soares_NhHMPu_S1; Soares_fetal_liver_spleen_1NFLS_S1;

15 Soares_multiple_sclerosis_2NbHMSP; NCI_CGAP_GCB1; Human Osteoclastoma; T cell helper II; Human Cardiomyopathy, subtracted; HUMAN STOMACH; Soares_NhHMPu_S1; Synovial Fibroblasts (III/TNF), sub; Human Prostate; KMH2; Apoptotic T-cell; Stratagene NT2 neuronal precursor 937230; Hepatocellular Tumor, re-excision; Soares_NFL_T_GBC_S1; Stratagene hNT neuron (#937233); PC3

20 Prostate cell line; Human Testes, Reexcision; Endothelial-induced; Human fetal heart, Lambda ZAP Express; Human Microvascular Endothelial Cells, fract. A; Human Endometrial Tumor; Hodgkin's Lymphoma II; Osteoblasts; Nine Week Old Early Stage Human; NCI_CGAP_GC4; NCI_CGAP_Lym6; Soares fetal liver spleen 1NFLS and Stratagene HeLa cell s3 937216.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 64 as residues: Gln-28 to Gly-36. Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1744 of SEQ ID NO:15, b is an integer of 15 to 1758, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

15

FEATURES OF PROTEIN ENCODED BY GENE NO: 6

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain INIB and to a lesser extent in normalized infant brain cDNA; Infant brain, Bento Soares; Human Fetal Brain; Human Fetal Brain; Soares melanocyte 2NbHM; Early Stage Human Brain; NCI_CGAP_Lu5; Human 8 Week Whole Embryo; Nine Week Old Early Stage Human; Tongue carcinoma; H. Striatum Depression, subt; Frontal lobe,dementia,re-excision; Raji Cells, cyclohexamide treated; Human Hypothalamus,schizophrenia, re-excision; Human Synovium; Soares_total_fetus_Nb2HF8_9w; Alzheimers, spongy change; Human Amygdala,re-excision; Human Osteosarcoma; Stromal cell TF274; 12 Week Old Early Stage Human; Human Substantia Nigra; Brain frontal cortex; Human

25

Synovial Sarcoma; Activated T-Cell (12hs)/Thiouridine labelledEco; Human Amygdala; Smooth muscle,control; H. Frontal cortex,epileptic,re-excision; Gessler Wilms tumor; Human colorectal cancer; Soares_NFL_T_GBC_S1; Stratagene schizo brain S11; Activated T-cell(12h)/Thiouridine-re-excision; Soares_NhHMPu_S1 and
 5 Keratinocyte.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 65 as residues: Trp-19 to Ala-24, Gly-32 to Met-39. Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly
 10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
 15 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1887 of SEQ ID NO:16, b is an integer of 15 to 1901, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 7

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares_pregnant_uterus_NbHPU and to a lesser extent in Soares_placenta_8to9weeks_2NbHP8to9W; Fetal Heart, re-excision;
 25 Soares_NhHMPu_S1; Soares_NFL_T_GBC_S1; normalized infant brain cDNA; Soares melanocyte 2NbHM; Human Bone Marrow, treated; Normal Prostate;

NCI_CGAP_Pr2; NCI_CGAP_Pr3; NCI_CGAP_Pr12; NCI_CGAP_Pr25;
 STRATAGENE Human skeletal muscle cDNA library, cat. #936215.;
 Soares_NhHMPu_S1; Soares_testis_NHT; Soares_total_fetus_Nb2HF8_9w; Human
 Pre-Differentiated Adipocytes; Weizmann Olfactory Epithelium;

- 5 Soares_NSF_F8_9W_OT_PA_P_S1; metastatic squamous cell lung carcinoma,
 poorly differentiated; Healing Abdomen wound, 70&90 min post incision; Activated
 T-cells; Stanley Frontal SB pool 1; Papillary serous cystic neoplasm of low malignant
 potential (ovary); Soares_testis_NHT; Human T-cell lymphoma, re-excision; Human
 Normal Breast; Synovial hypoxia; Human Hypothalamus, Schizophrenia; Human
- 10 Adipose; CHME Cell Line, treated 5 hrs; STRATAGENE Human skeletal muscle
 cDNA library, cat. #936215.; Soares_pregnant_uterus_NbHPU; Fetal Heart; CHME
 Cell Line, untreated; Soares_fetal_liver_spleen_1NFLS_S1; Smooth muscle, serum
 treated; Adipocytes; Bone marrow; NCI_CGAP_Pr28; H. Frontal cortex, epileptic, re-
 excision; Osteoblasts; T cell helper II and Primary Dendritic Cells, lib 1.

- 15 Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are
 related to SEQ ID NO:17 and may have been publicly available prior to conception of
 the present invention. Preferably, such related polynucleotides are specifically
 excluded from the scope of the present invention. To list every related sequence
- 20 would be cumbersome. Accordingly, preferably excluded from the present invention
 are one or more polynucleotides comprising a nucleotide sequence described by the
 general formula of a-b, where a is any integer between 1 to 3131 of SEQ ID NO:17, b
 is an integer of 15 to 3145, where both a and b correspond to the positions of
 nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a
- 25 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 8

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

- 5 gi|868189 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "No definition line found [Caenorhabditis elegans]". A partial alignment demonstrating the observed homology is shown immediately below.

```

10  >gi|868189 No definition line found [Caenorhabditis elegans]
    >sp|Q19202|Q19202
        COSMID F08C6.
        Length = 296

15      Plus Strand HSPs:

        Score = 758 (266.8 bits), Expect = 1.6e-74, P = 1.6e-74
        Identities = 149/294 (50%), Positives = 199/294 (67%), Frame = +2

20      Q:  176 VIADLDTESRAQEENTWFSYLKKGylTLSDSGDKVAVEWDKDHGV-LESHLAEKGRGMEL 352
           +I DLD +S+ + W S + +G+L +S + +DKD +++++A GR MEL
           S:  2  LITDLHDHDSK--DGKKWKSLSVSRGFLKVSADHKHADIHFDDSEYYVDNTIAAGGRAMEL 59

25      Q:  353 SDLIVFNGKLYSVDVRTGVVYQIEGSKAVPWPVILSDGDTGTEVGKFAEWLAVKDERLYVG 532
           SDL VFNGKLYS+DDRTG++YQI KA+PWV+L+DG G V KGFK EW+ VKD L VG
           S:  60  SDLAVFNGKLYSIDDRTGLIYQISDKKALPWLNDGPGNVVRGFKGEWITVKDTEILIVG 119

           Q:  533 GLGKEWTTTGDVVNENPEWVKVVGKSGVDHENWVSNYNALRAAGIQPPGYLIHESAC 712
           GLGKEWTTT G VN++P WVK V G+V HENW Y +R AAGI+ PGY+IHE+
           S:  120 GLGKEWTTTGDVYVNDHPMVVKHVSAGHAVHHENWKDVYIRVRAAGIEYPGYMIHEAVQ 179

30      Q:  713 WSDTLQRWFFLPRRASQERYSEKDDERKGANLLLSASPFDGDIASVHVGAVV-PTHGFSS 889
           WS ++WFFLPRR S E+YSE +DE +G N+L+ + + D V VG+ + GF++
           S:  180 WSAIHRKWFFLPRRMSNEKYSEADENRGTNVLVIGNEELTDFEVVRVGSNNKSRGF 239

35      Q:  890 FKFIPNTDDQIIVALKSEEDSGR-VASYIMAFTLDRGFLLPETKI-GSVKYEGIEF 1051
           F+F+PNT Q+IVA+KSEE G+ VASY F + G +L E + G KYEGI F
           S:  240 FQFVFNTHHQLIVAIAKSEEDGKFPVASYASVFDTHGNVILDEYLLHGPYKYGIAF 295
  
```

- 40 The segment of gi|868189 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 113. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities

are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

- Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 114 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares placenta Nb2HP and to a lesser extent in Human Endometrial Tumor; Soares ovary tumor NbHOT; Human Whole Six Week Old Embryo; Colon Tumor II; Soares melanocyte 2NbHM; Human Cerebellum; Jurkat T-Cell, S phase; Human Hippocampus; Stratagene pancreas (#937208); Normal colon; Human Microvascular Endothelial Cells, fract. A; Activated T-cell(12h)/Thiouridine-re-excision; Soares_NFL_T_GBC_S1; Stratagene fetal retina 937202; Pharynx carcinoma; Stratagene placenta (#937225); Stratagene HeLa cell s3 937216; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Soares_multiple_sclerosis_2NbHMSP; Ovarian Tumor 10-3-95; Soares_NhHMPu_S1; Smooth muscle, serum induced, re-exc; Macrophage-oxLDL, re-excision; NCI_CGAP_Pr12; Fetal Heart; PC3 Prostate cell line; Stratagene pancreas (#937208); Smooth muscle, serum treated; Human Fetal Heart; Activated T-Cell (12hs)/Thiouridine labelled Eco; Bone Marrow Cell Line (RS4,11); NCI_CGAP_Br2; NCI_CGAP_Co2; NCI_CGAP_GC4; NCI_CGAP_Co10; NCI_CGAP_Co11; NCI_CGAP_GCB1; Osteoblasts; Soares fetal liver spleen INFLS; Heart; Activated T-Cells, 12hrs, differentially expressed;
- Soares_NhHMPu_S1; Soares_testis_NHT; Soares_fetal_liver_spleen_INFLS_S1; normalized infant brain cDNA; Human Amygdala Depression, re-excision; Activated

- T-Cells, 12 hrs, subtracted; Human rejected kidney; Tongue Normal; Human Microvascular Endothelial Cells, fract. B; Human OB HOS treated (1 nM E2) fraction I; Human Colon; Human colon carcinoma (HCC) cell line, remake; eosinophil-IL5 induced; Human Fetal Spleen; Human Pituitary, subtracted; human colon cancer;
- 5 Human Normal Breast; STROMAL -OSTEOCLASTOMA; NTERA2 + retinoic acid, 14 days; Amniotic Cells - Primary Culture; Human endometrial stromal cells-treated with progesterone; Human Osteosarcoma; Human endometrial stromal cells; Human Colon, re-excision; Human Pituitary, subt IX; wilm's tumor; Brain Frontal Cortex, re-excision; Human Neutrophil; Human Prostate; Human Bone Marrow, re-excision;
- 10 Breast Cancer Cell line, angiogenic; L428; Human Fetal Kidney; human ovarian cancer; Human Uterine Cancer; Soares_placenta_8to9weeks_2NbHP8to9W; T-Cell PHA 24 hrs; Macrophage-oxLDL; Human Pancreas Tumor, Reexcision; Olfactory epithelium,nasalcavity; Human Fetal Brain; Soares_fetal_lung_NbHL19W; Human adult testis, large inserts; Hepatocellular Tumor, re-excision; Stratagene colon
- 15 (#937204); Stratagene neuroepithelium (#937231); NCI_CGAP_Lu5; Resting T-Cell Library,II; 12 Week Old Early Stage Human; Colon Carcinoma; Human Placenta; Human Synovial Sarcoma; Human Placenta; human tonsils; Soares_placenta_8to9weeks_2NbHP8to9W; Endothelial-induced; Human Primary Breast Cancer Reexcision; Colon Normal III; Soares_fetal_heart_NbHH19W;
- 20 Monocyte activated; Human Bone Marrow, treated; Spleen, Chronic lymphocytic leukemia; T Cell helper I; Human Testes; Soares_fetal_lung_NbHL19W; H. Frontal cortex,epileptic,re-excision; NCI_CGAP_Co9; NCI_CGAP_GC3; NCI_CGAP_Pr8; NCI_CGAP_Pr11; NCI_CGAP_Pr12; NCI_CGAP_Pr22; NCI_CGAP_Pr25; NCI_CGAP_Sch1; Human 8 Week Whole Embryo; Nine Week Old Early Stage
- 25 Human and T cell helper II.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 67 as residues: Pro-27 to Lys-34. Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2954 of SEQ ID NO:18, b is an integer of 15 to 2968, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 9

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi|204412 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "glutaminase [Rattus norvegicus]". A partial alignment demonstrating the observed homology is shown immediately below.

```

>gi|204412 glutaminase [Rattus norvegicus] >sp|P13264|GLSK_RAT
GLUTAMINASE,
      KIDNEY ISOFORM PRECURSOR (EC 3.5.1.2) (GLS) (L-GLUTAMINE
      AMIDOHYDROLASE). >bbs|174849 glutaminase, GA {C-terminal}
[swine,
```

LLC-PK1-F+ cells, Peptide Mitochondrial Partial, 175 aa [Sus
scrofa] [SUB 500-674]
Length = 674

5 Plus Strand HSPs:

Score = 2299 (809.3 bits), Expect = 8.9e-238, P = 8.9e-238
Identities = 433/438 (98%), Positives = 437/438 (99%), Frame = +1

10 Q: 4 DAFNGSEGKELVASGENNIKQGLLPSEDLFFYTTIAEGQEKIPVHKFITALKSTGLRTSD 183
DAFNGSEGKE+VA+G+NK+KQGLLPSEDLFFYTTIAEGQEKIPVHKFITALKSTGLRTSD
S: 118 DAFNGSEGKEMVAAGDNKVKQGLLPSEDLFFYTTIAEGQEKIPVHKFITALKSTGLRTSD 177

15 Q: 184 PRLKECDMLRLTLQTSSDGVMLDKDLFKKCVQSNIVLLTQAFRRKFVIPDFMSFTSHID 363
PRLKECDMLRLTLQTSSDGVMLDKDLFKKCVQSNIVLLTQAFRRKFVIPDFMSFTSHID
S: 178 PRLKECDMLRLTLQTSSDGVMLDKDLFKKCVQSNIVLLTQAFRRKFVIPDFMSFTSHID 237

Q: 364 ELYESAKKQSGGKVADYIPQAKFSPDLWGVSVCTVDGQRHSTGDTKVPFCLQSCVKPLK 543
ELYESAKKQSGGKVADYIPQAKFSPDLWGVSVCTVDGQRHS GDTKVPFCLQSCVKPLK
20 S: 238 ELYESAKKQSGGKVADYIPQAKFSPDLWGVSVCTVDGQRHSIGDTKVPFCLQSCVKPLK 297

Q: 544 YAIAVNDLGTEYVHRVYVGKEPSGLRFNKLFLNEDDKPHNPMVNAGAIVVTSLIKQGVNNA 723
YAIAVNDLGTEYVHRVYVGKEPSGLRFNKLFLNEDDKPHNPMVNAGAIVVTSLIKQGVNNA
S: 298 YAIAVNDLGTEYVHRVYVGKEPSGLRFNKLFLNEDDKPHNPMVNAGAIVVTSLIKQGVNNA 357

25 Q: 724 EKFDYVMQFLNKMAGNEYVGFNSNATFQSERESGDRNFAIGYILKEKKCFPEGDMVGILD 903
EKFDYVMQFLNKMAGNEYVGFNSNATFQSERESGDRNFAIGYILKEKKCFPEGDMVGILD
S: 358 EKFDYVMQFLNKMAGNEYVGFNSNATFQSERESGDRNFAIGYILKEKKCFPEGDMVGILD 417

30 Q: 904 FFFQLCSIEVTCESASVMAATLANGGFCPITGERVLSPEAVRNTLSLMHSCGMDFSGQF 1083
FFFQLCSIEVTCESASVMAATLANGGFCPITGERVLSPEAVRNTLSLMHSCGMDFSGQF
S: 418 FFFQLCSIEVTCESASVMAATLANGGFCPITGERVLSPEAVRNTLSLMHSCGMDFSGQF 477

Q: 1084 AFHVGLPAKSGVAGGILLVVPNVGMCMCWSPPDLKMGNSVKGIFHCHDLVSLCNFHNND 1263
AFHVGLPAKSGVAGGILLVVPNVGMCMCWSPPDLKMGNSVKGIFHCHDLVSLCNFHNND
35 S: 478 AFHVGLPAKSGVAGGILLVVPNVGMCMCWSPPDLKMGNSVKGIFHCHDLVSLCNFHNND 537

Q: 1264 LRHFACKLDPRREGGDQR 1317
LRHFACKLDPRREGGDQR
40 S: 538 LRHFACKLDPRREGGDQR 555

The segment of gil204412 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 115. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 116 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- 5 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: NCI_CGAP_GCB1 and to a lesser extent in T-Cell PHA 24 hrs; 12 Week Old Early Stage Human; Hodgkin's Lymphoma II; Human 8 Week Whole Embryo; Soares placenta Nb2HP; Primary Dendritic Cells, lib 1; Mo7e Cell Line GM-CSF treated (1ng/ml); Human Eosinophils; Colon Carcinoma; 12 Week
- 10 Early Stage Human II, Reexcision; Human T-cell Lymphoma, re-excision; Stratagene endothelial cell 937223; Stratagene NT2 neuronal precursor 937230; Bone Marrow Stromal Cell, untreated; Stratagene lung (#937210); Soares_pregnant_uterus_NbHPU; Human Endometrial Tumor; Human fetal heart, Lambda ZAP Express; Osteoblasts; Human Cerebellum; C7MCF7 cell line, estrogen
- 15 treated; Soares_placenta_8to9weeks_2NbHP8to9W; Bone Marrow Stroma, TNF&LPS ind; Larynx Normal; Human Microvascular Endothelial Cells, fract. B; Activated T-Cells, 8 hrs, subtracted; Thymus; Dermatofibrosarcoma Protuberance; Human Umbilical Vein Endothelial Cells, fract. A; Human Pancreatic Carcinoma; Human Thyroid; B Cell lymphoma; Human Synovium; pBMC stimulated w/ poly
- 20 I/C; Synovial hypoxia-RSF subtracted; Healing groin wound, 7.5 hours post incision; NCI_CGAP_GCB1; Human endometrial stromal cells-treated with progesterone; Soares_pregnant_uterus_NbHPU; Human Osteoclastoma, re-excision; Jurkat T-cell G1 phase; Healing groin wound, 6.5 hours post incision; Synovial Fibroblasts (III/TNF), subt; Human Chronic Synovitis; Soares_fetal_heart_NbHH19W; Human
- 25 Umbilical Vein, Reexcision; Human Bone Marrow, re-excision; TF-1 Cell Line GM-CSF Treated; KMH2; human ovarian cancer; 12 Week Old Early Stage Human, II;

- Human Umbilical Vein Endothelial Cells, uninduced;
 Soares_placenta_8to9weeks_2NbHP8to9W; Human umbilical vein endothelial cells,
 IL-4 induced; Human Activated Monocytes; Human fetal heart, Lambda ZAP
 Express; Human retina cDNA randomly primed sublibrary; NCI_CGAP_GCB1;
- 5 Human Testes Tumor, re-excision; Human Adrenal Gland Tumor; Rejected Kidney,
 lib 4; Ovarian Tumor 10-3-95; Soares_fetal_liver_spleen_1NFLS_S1; NTERA2,
 control; Gessler Wilms tumor; Liver HepG2 cell line.; PC3 Prostate cell line; Resting
 T-Cell Library,II; Human T-Cell Lymphoma; NCI_CGAP_GCB1; Human Substantia
 Nigra; Colon Tumor II; Human fetal lung; Human Placenta; Human Fetal Heart;
- 10 Human Neutrophil, Activated; Endothelial-induced; Anergic T-cell; HUMAN B
 CELL LYMPHOMA; Human Bone Marrow, treated; NCI_CGAP_Sch1 and Nine
 Week Old Early Stage Human.

- Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are
- 15 related to SEQ ID NO:19 and may have been publicly available prior to conception of
 the present invention. Preferably, such related polynucleotides are specifically
 excluded from the scope of the present invention. To list every related sequence
 would be cumbersome. Accordingly, preferably excluded from the present invention
 are one or more polynucleotides comprising a nucleotide sequence described by the
- 20 general formula of a-b, where a is any integer between 1 to 3939 of SEQ ID NO:19, b
 is an integer of 15 to 3953, where both a and b correspond to the positions of
 nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a
 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 10

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

5 gil2731377 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "similar to alcohol dehydrogenase/ribitol dehydrogenase [*Caenorhabditis elegans*]" . A partial alignment demonstrating the observed homology is shown immediately below.

```

10  >gi|2731377 similar to alcohol dehydrogenase/ribitol dehydrogenase
    [Caenorhabditis elegans] >sp|Q09979|Q09979 HYPOTHETICAL 105.9
KD
    PROTEIN C17G10.8 IN CHROMOSOME II.
    Length = 938

15      Minus Strand HSPs:

    Score = 956 (336.5 bits), Expect = 1.8e-95, P = 1.8e-95
    Identities = 192/466 (41%), Positives = 287/466 (61%), Frame = -3

20      Q: 1664 VIDQKVYEIQDIYENSWKLTFRFFKNTWPPEAEAIAPQVG-NDAVFLILYKELYRHIY 1488
        V +Q V EI +Y+ ++ LTERFF++ WP+ + +G + +F+ILYKELYR +Y
        S: 45 VDEQNVPEILILYDQAFPDLTFRFFDRMWPDENVVERIIGPGNKLFIILYKELYRQLY 104

25      Q: 1487 AKVSGGSLEQRFESYYNYCNLFNYILNADGPAPLELPNQWLNDIIDEFIYQFQSFQSYR 1308
        A+ + GP L R+ES+ NY LF+ +L++ P PL LPN WLNDIIDEF+YQFQ+F Y+
        S: 105 ARNTRGELLVHRYESFMNYQLFSELLSSKDIPLSLPNVWLNDIIDEFVYQFQAFCLYK 164

30      Q: 1307 CKTAKKSEEEIDFL---RSNPKIWXXXXXXXXXXXXKSNINRQLEVYTSGGDPESVAG 1137
        K++ +E++ L N W KS I QL+ +P+SVA
        S: 165 ANPGKRNADEVEDLINEENQNAWNIYPVLNILYLSKFSQIVEQLKALKEKRNPFDSVAD 224

        Q: 1136 EYGRHSLYKMLGYFXXXXXXXXXXXXDYQAIKVLNIELNKKSMYSRVPECQVTTY 957
        E+G+ LY LGYF DY+QA+K ++ +++ K +Y+ VP C VT +Y+
35      S: 225 EFGQSDLYFKLGYFALIGLLRTHVLLGDYHQAQKTVQYVDIDPKGIYNTVPTCLVTLHYF 284

        Q: 956 VGFAYLMRRYQDAIRVFANILLIYQRTKSMFQ---RTTYKYMINKNEQMHALLAIA 789
        VGF++LMMR Y A +F M LLYIQRKTS+ + ++Y+I K +Q+ LLAI
        S: 285 VGFSLMMRNIGEATKMFVNCILLYIQRKTSVQNQPSKRNQFYDVIGKTWQDLFHLLAIC 344

40      Q: 788 LTHYPRIDESIHLQLREKYGDKMLRMQKGDPOVYEELFYSYCPKFLSPVVPYNDVNHVN 609
        L + P RIDESI QL E+ G++M+ M G+ + F+ CPKFLSP Y+ V N
        S: 345 LAIQPRIDESIASQLSERCGERMMHMANGNIDEFRNAPATGCPKFLSPTTVVYEGV--N 402

45      Q: 608 YHKEFLQQLKVFSDEVOOQAQLSTIRSFLKLYTTPVAKLAGFLDLTRQEFRI---QLL 438
        KEP L+Q + F + ++ Q L +R +LKLYTT+P KLA F+D+ ++ +LL
        S: 403 QSKPELLRQTQSFLGIESQHALPVLRLGYLKLYTTLPTTKKLASFHDVDDENHYDSFIQKLL 462

```


Q: 437 VFHKMKNLVWTSGLSALDGEFQSASEVDFYIDKDMIHIADTKVARRYG 291
 +K + L +G S+ D + + ++DFY+D+DMI+IADTKVAR G
 S: 463 TYKMIVNELGKEAGPSSADDD-EPQTDIDFYVDRDMINTADTKVARHVG 510

5

The segment of gil2731377 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 117. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein.

- 10 Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 118 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human 8 Week Whole Embryo and to a lesser extent in Human Testes Tumor; Osteoblasts; T cell helper II; Bone Marrow Cell Line (RS4,11); Stratagene lung (#937210); Early Stage Human Brain; Human Synovial
- 20 Sarcoma; NCI_CGAP_GCB1; Human Amygdala; Soares fetal liver spleen 1NFLS; Soares melanocyte 2NbHM; Human Testes, Reexcision; Human Endometrial Tumor; Keratinocyte; Nine Week Old Early Stage Human; Primary Dendritic Cells, lib 1; Fetal Heart; Human Adult Pulmonary, re-excision; Spleen, Chronic lymphocytic leukemia; Human Fetal Dura Mater; Soares_fetal_liver_spleen_1NFLS_S1;
- 25 Adipocytes; Human Fetal Heart; Human Testes; Activated T-cell(12h)/Thiouridine-re-excision; NCI_CGAP_Co3; Jurkat T-Cell, S phase; Human Chronic Synovitis; Human umbilical vein endothelial cells, IL-4 induced; Hemangiopericytoma; Macrophage (GM-CSF treated); Smooth muscle, serum induced, re-exc;

- Soares_NhHMPu_S1; CHME Cell Line, untreated; Colon Carcinoma; Human Fetal Kidney, Reexcision; Human Fetal Lung III; CD34 depleted Buffy Coat (Cord Blood), re-excision; Colon Normal III; HUMAN B CELL LYMPHOMA; Human Bone Marrow, treated; NCI_CGAP_AA1; NCI_CGAP_Pr21; Stratagene HeLa cell s3
- 5 937216; Stratagene NT2 neuronal precursor 937230; Human Cerebellum; Soares infant brain INIB; Soares ovary tumor NbHOT; Smooth Muscle- HASTE normalized; Glioblastoma; Jurkat T-cell G1 phase; H. Kidney Medulla, re-excision; KMH2; Apoptotic T-cell; Human Fetal Kidney; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Spinal cord; Human Testes Tumor, re-excision;
- 10 Soares_fetal_heart_NbHH19W; NCI_CGAP_Lu5; Gessler Wilms tumor; Stratagene liver (#937224); Human T-Cell Lymphoma; 12 Week Early Stage Human II, Reexcision; Human Placenta; Bone marrow; Human Neutrophil, Activated; Endothelial-induced; Soares_fetal_lung_NbHL19W; Soares_senescent_fibroblasts_NbHSF; NCI_CGAP_Lu5; NCI_CGAP_Kid3; H.
- 15 Frontal cortex, epileptic, re-excision; NCI_CGAP_Co10; NCI_CGAP_GCB1; NCI_CGAP_Pr10; Soares_fetal_lung_NbHL19W; Soares_fetal_heart_NbHH19W; Stratagene pancreas (#937208); Stratagene colon HT29 (#937221); Stratagene corneal stroma (#937222); Stratagene ovarian cancer (#937219); Stratagene neuroepithelium (#937231); NCI_CGAP_Pr28; Human Uterus, normal; Normal Ovary - 9805C040R;
- 20 Weizmann Olfactory Epithelium; Saos2 Cells, Untreated; Human (Caco-2) cell line, adenocarcinoma, colon, remake; HSC172 cells; Healing Abdomen wound, 70&90 min post incision; Human Thyroid; Human Liver; Human Soleus; Stratagene ovary (#937217); Human Pineal Gland; Human Lung Cancer, re-excision; NCI_CGAP_CLL1; HEL cell line; STROMAL -OSTEOCLASTOMA; Stratagene
- 25 placenta (#937225); Human Adipose Tissue, re-excision; Stratagene neuroepithelium NT2RAM1 937234; Human endometrial stromal cells; Stratagene muscle 937209;

- Stratagene endothelial cell 937223; Myeloid Progenitor Cell Line; wilm's tumor; T-Cell PHA 16 hrs; TF-1 Cell Line GM-CSF Treated; Stratagene fetal spleen (#937205); 12 Week Old Early Stage Human, II; Human Umbilical Vein Endothelial Cells, uninduced; Human Pancreas Tumor; Merkel Cells; Human
- 5 Hypothalamus, Schizophrenia; Human Pancreas Tumor, Reexcision; Human Thymus; Stratagene neuroepithelium (#937231); Human Adrenal Gland Tumor; Ovarian Tumor 10-3-95; NTERA2, control; Soares_NbHFB; Soares_testis_NHT; Pancreas Islet Cell Tumor; NCI_CGAP_Alv1; Soares_NhHMPu_S1; Colon Tumor; 12 Week Old Early Stage Human; Colon Normal II; Human Placenta; H Macrophage (GM-
- 10 CSF treated), re-excision; Dendritic cells, pooled; Normal colon; Soares_multiple_sclerosis_2NbHMSP; Gessler Wilms tumor; Heart; Human Osteoclastoma; Anergic T-cell; Soares_fetal_heart_NbHH19W; Human Microvascular Endothelial Cells, fract. A; Monocyte activated; NCI_CGAP_GC4; NCI_CGAP_Co12; NCI_CGAP_Pr1; NCI_CGAP_Alv1; NCI_CGAP_Pr23;
- 15 NCI_CGAP_Thy1; Stratagene hNT neuron (#937233); Soares placenta Nb2HP; H. Umbilical Vein Endothelial Cells, IL4 induced; Human Adult Spleen, subtracted; Human Fetal Kidney; Soares_parathyroid_tumor_NbHPA; Human Astrocyte; Stratagene fibroblast (#937212); Skin, burned; Human Adult Spleen, fraction II; H. hypothalamus, frac A, re-excision; Human Old Ovary; Soares_NhHMPu_S1; Larynx
- 20 tumor; Colon, normal; Thyroid Normal (SDCA2 No); Stromal Cells; Human Adult Heart; CD34+ cell, I, frac II; Normal Ovary, Premenopausal; Human osteoarthritis, fraction I; NCI_CGAP_Pan1; B-cells (stimulated); Colorectal Tumor; Human 8 Week Whole Embryo, subtracted; Human Colon Cancer, subtracted; NCI_CGAP_Brn23; Human Pituitary, re-excision; L1 Cell line; Human Adult Spleen;
- 25 Human Colon; Human OB HOS treated (10 nM E2) fraction I; Human Cerebellum, subtracted; Human Colon Carcinoma (HCC) cell line; Human Umbilical Vein

- Endothelial Cells, fract. A; Fetal Heart, re-excision; Supt Cells, cyclohexamide treated; Human Fetal Bone; Human Quadriceps; Human T-cell lymphoma, re-excision; HSA 172 Cells; Human Skin Tumor; Human Normal Breast; Mesangial cell, frac 2; NCI_CGAP_Ut1; NCI_CGAP_Ut2; Dendritic Cells From CD34 Cells;
- 5 Hepatocellular Tumor, re-excision; Human Prostate Cancer, Stage C fraction; pBMC stimulated w/ poly I/C; Breast Cancer cell line, MDA 36; Smooth muscle, IL1b induced; Human Stomach, re-excision; Human Frontal Cortex, Schizophrenia; Human Amygdala, re-excision; HL-60, PMA 4H, re-excision; Pancreas normal PCA4 No; Soares_NhHMPu_S1; Stratagene ovarian cancer (#937219); Synovial hypoxia;
- 10 Prostate BPH; Brain Frontal Cortex, re-excision; Human Adult Small Intestine; Spinal Cord, re-excision; Human Umbilical Vein, Reexcision; Human Bone Marrow, re-excision; Human Thymus; Human Brain, Striatum; Monocyte activated, re-excision; Human Osteoblasts II; Soares_fetal_lung_NbHL19W; Human Uterine Cancer; Human Adult Testes, Large Inserts, Reexcision; Human Heart; Stromal cell
- 15 TF274; Olfactory epithelium, nasalcavity; Liver, Hepatoma; Human Placenta (re-excision); Ulcerative Colitis; Human fetal heart, Lambda ZAP Express; Human retina cDNA randomly primed sublibrary; NCI_CGAP_Lu5; NCI_CGAP_Kid6; Stratagene muscle 937209; Soares_fetal_liver_spleen_1NFLS_S1; Bone Marrow Stromal Cell, untreated; Human Fetal Brain; Human Thymus Stromal Cells; Rejected Kidney, lib 4;
- 20 CHME Cell Line, treated 5 hrs; Human Whole Six Week Old Embryo; Fetal Liver, subtraction II; NCI_CGAP_GC4; NCI_CGAP_Ut1; NCI_CGAP_Ut3; NCI_CGAP_CLL1; NCI_CGAP_Kid8; Barstead spleen HPLRB2; Soares_NhHMPu_S1; Stratagene fetal retina 937202; Stratagene lung carcinoma 937218; Hepatocellular Tumor, re-excision; Human Liver, normal; Macrophage-
- 25 oxLDL, re-excision; Human Gall Bladder; NCI_CGAP_Pr25; PC3 Prostate cell line; Human Ovarian Cancer Reexcision; Resting T-Cell Library, II; Human Substantia

Nigra; breast lymph node cDNA library; Soares breast 3NbHBst; Colon Tumor II;
 Human retina cDNA randomly primed sublibrary; Infant brain, Bento Soares;
 Soares_NhHMPu_S1; Soares_NFL_T_GBC_S1;
 Soares_fetal_liver_spleen_INFLS_S1; Soares_senescent_fibroblasts_NbHSF;

- 5 Stratagene lung carcinoma 937218; Primary Dendritic cells,frac 2; Gessler Wilms tumor; human tonsils; Activated T-Cell (12hs)/Thiouridine labelledEco;
 NCI_CGAP_Ut1; NCI_CGAP_Ut2; NCI_CGAP_Pan1; Soares_testis_NHT;
 Soares_senescent_fibroblasts_NbHSF; Human Primary Breast Cancer Reexcision;
 Soares_placenta_8to9weeks_2NbHP8to9W; NCI_CGAP_GCB1; Smooth
 10 muscle,control; NCI_CGAP_Alv1; NCI_CGAP_Kid6; NCI_CGAP_Lei2;
 NCI_CGAP_Pr12; NCI_CGAP_Pr25; T Cell helper I; Hodgkin's Lymphoma II;
 NCI_CGAP_Br3; NCI_CGAP_Co1; NCI_CGAP_Co2; NCI_CGAP_Co9;
 NCI_CGAP_GC5; NCI_CGAP_Pr4; NCI_CGAP_HSC1; NCI_CGAP_Lar1;
 Stratagene muscle 937209 and Stratagene endothelial cell 937223.

- 15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
 20 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1949 of SEQ ID NO:20, b is an integer of 15 to 1963, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a

FEATURES OF PROTEIN ENCODED BY GENE NO: 11

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares fetal liver spleen 1NFLS and to a lesser extent in
- 5 Soares_fetal_heart_NbHH19W; NCI_CGAP_GCB1; Soares melanocyte 2NbHM; Colon Normal III; Soares breast 2NbHBst; Soares breast 3NbHBst; Human Microvascular Endothelial Cells, fract. A; Activated T-cell(12h)/Thiouridine-re-excision; Stratagene lung (#937210); Smooth muscle, serum induced,re-exc; 12 Week Early Stage Human II, Reexcision; Activated T-Cell (12hs)/Thiouridine labelledEco;
 - 10 Soares_pregnant_uterus_NbHPU; Human Adult Heart,re-excision; Stratagene fetal spleen (#937205); Human Placenta (re-excision); Human Testes Tumor, re-excision; Fetal Liver, subtraction II; Soares_fetal_liver_spleen_1NFLS_S1; Clontech human aorta polyA+ mRNA (#6572); Dendritic cells, pooled; Human Fetal Lung III; Endothelial-induced; Soares_fetal_liver_spleen_1NFLS_S1; Stratagene pancreas
 - 15 (#937208); Spleen, Chronic lymphocytic leukemia; NCI_CGAP_Lu5; NCI_CGAP_Ov2; Soares_parathyroid_tumor_NbHPA; Nine Week Old Early Stage Human; Human Kidney Medulla - unamplified; Human Prostate, subtracted; Human (HCC) cell line liver (mouse) metastasis, remake; Human Cardiomyopathy, subtracted; Adipocytes,re-excision; human colon cancer; H. cerebellum, Enzyme
 - 20 subtracted; Human Soleus; LPS activated derived dendritic cells; Cem cells cyclohexamide treated; NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Healing groin wound - zero hr post-incision (control); Synovial hypoxia-RSF subtracted; Human endometrial stromal cells; H. Lymph node breast Cancer; Brain Frontal Cortex, re-excision; Human Adult Small Intestine; Human Neutrophil;
 - 25 Human Chronic Synovitis; Human Umbilical Vein Endothelial Cells, uninduced; Human Hippocampus; Human Adipose; Human Activated Monocytes; Human

Rhabdomyosarcoma; NCI_CGAP_GCB1; Soares_NFL_T_GBC_S1;
 Soares_fetal_heart_NbHH19W; Soares_parathyroid_tumor_NbHPA; Hepatocellular
 Tumor, re-excision; Soares_fetal_liver_spleen_INFLS_S1; Fetal Heart; PC3 Prostate
 cell line; Human Eosinophils; Human Synovial Sarcoma; Neutrophils control, re-
 5 excision; Soares_senescent_fibroblasts_NbHSP; human tonsils; Human Fetal Heart;
 Endothelial cells-control; Anergic T-cell; Human Amygdala; HUMAN B CELL
 LYMPHOMA; NCI_CGAP_Co3; NCI_CGAP_Ew1; NCI_CGAP_Pr1;
 NCI_CGAP_Kid3; Human 8 Week Whole Embryo; T cell helper II and Human
 Cerebellum.

- 10 Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are
 related to SEQ ID NO:21 and may have been publicly available prior to conception of
 the present invention. Preferably, such related polynucleotides are specifically
 excluded from the scope of the present invention. To list every related sequence
 15 would be cumbersome. Accordingly, preferably excluded from the present invention
 are one or more polynucleotides comprising a nucleotide sequence described by the
 general formula of a-b, where a is any integer between 1 to 3031 of SEQ ID NO:21, b
 is an integer of 15 to 3045, where both a and b correspond to the positions of
 nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a
 20 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 12

It has been discovered that this gene is expressed primarily in the following
 tissues/cDNA libraries: breast lymph node CDNA library.

- 25 Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 432 of SEQ ID NO:22, b is an integer of 15 to 446, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

10

FEATURES OF PROTEIN ENCODED BY GENE NO: 13

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

15 gi|206886 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "homologue to sec61 [Rattus rattus]". A partial alignment demonstrating the observed homology is shown immediately below.

20 >gi|206886 homologue to sec61 [Rattus rattus]
Length = 476

Plus Strand HSPs:

25 Score = 1770 (623.1 bits), Expect = 4.0e-215, Sum P(2) = 4.0e-215
Identities = 349/356 (98%), Positives = 349/356 (98%), Frame = +1

Q: 163 IKFLEVIKPFVCVILPEIQKPERKIQFKEKVLMTAITLFIPLVCCQIPLFGIMSSDSADPF 342
IKFLEVIKPFVCVILPEIQKPERKIQFKEKVLMTAITLFIPLVCCQIPLFGIMSSDSADPF

30 S: 3 IKFLEVIKPFVCVILPEIQKPERKIQFKEKVLMTAITLFIPLVCCQIPLFGIMSSDSADPF 62

Q: 343 YWMRVILASNRGTLMELGISPIVTSGLIMQLLAGAKIIIEVGDTPKDRALFNGAQKLFGMI 522
YWMRVILASNRGTLMELGISPIVTSGLIMQLLAGAKIIIEVGDTPKDRALFNGAQKLFGMI

35 S: 63 YWMRVILASNRGTLMELGISPIVTSGLIMQLLAGAKIIIEVGDTPKDRALFNGAQKLFGMI 122

Q: 523 ITIGQSIYVMTGMYGDPSEMGAGICLLITIQLFVAGLIVLLDELLQKGYGLGSGISLF 702
 ITIGQSIYVMTGMYGDPSEMGAGICLLITIQLFVAGLIVLLDELLQKGYGLGSGISLF
 S: 123 ITIGQSIYVMTGMYGDPSEMGAGICLLITIQLFVAGLIVLLDELLQKGYGLGSGISLF 182
 5 Q: 703 IATNICETIVWKAFSPTTVNTGRGMEFEGAIIFHLLATRTDKVRALREAFYRQNLPNL 882
 IATNICETIVWKAFSPTTVNTGRGMEFEGAIIFHLLATRTDKVRALREAFYRQNLPNL
 S: 183 IATNICETIVWKAFSPTTVNTGRGMEFEGAIIFHLLATRTDKVRALREAFYRQNLPNL 242
 10 Q: 883 MNLIATIFVFAVVIYFQGFVRDLPKISARYRGQYNTYPIKLFYTSNIPILQSALVSNLY 1062
 MNLIATIFVFAVVIYFQGFVRDLPKISARYRGQYNTYPIKLFYTSNIPILQSALVSNLY
 S: 243 MNLIATIFVFAVVIYFQGFVRDLPKISARYRGQYNTYPIKLFYTSNIPILQSALVSNLY 302
 Q: 1063 VISQML SARFSGNLLVSLGTWSDTSSGGPARAYPVGGLCYILSPPMNSMTGTSP 1230
 VISQML SARFSGNLLVSLGTWSDTSSGGPARAYPVGGLCYILSP S S P
 15 S: 303 VISQML SARFSGNLLVSLGTWSDTSSGGPARAYPVGGLCYILSPPEFSGVLEDP 358
 Score = 338 (119.0 bits), Expect = 4.0e-215, Sum P(2) = 4.0e-215
 Identities = 68/68 (100%), Positives = 68/68 (100%), Frame = +3
 20 Q: 1200 MVHELNRYPITAAAFGGLCIGALSVLADFLGAIGSGTGILLAVTIIYQYFEIFVKEQSEV 1379
 MVHELNRYPITAAAFGGLCIGALSVLADFLGAIGSGTGILLAVTIIYQYFEIFVKEQSEV
 S: 409 MVHELNRYPITAAAFGGLCIGALSVLADFLGAIGSGTGILLAVTIIYQYFEIFVKEQSEV 468
 25 Q: 1380 GSMGALLF 1403
 GSMGALLF
 S: 469 GSMGALLF 476

The segments of g1206886 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 119 and SEQ ID NO. 121. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 120 and/or SEQ ID NO. 122 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Chondrosarcoma; Primary Dendritic Cells, lib 1 and to a lesser extent in Human Pancreas Tumor, Reexcision; Human Adult

- Pulmonary, re-excision; Human endometrial stromal cells-treated with estradiol; NCI_CGAP_GCB1; T Cell helper I; T cell helper II; Soares ovary tumor NbHOT; Human OB MG63 treated (10 nM E2) fraction I; Activated T-cells, 24 hrs, re-excision; TF-1 Cell Line GM-CSF Treated; Human Activated T-Cells; Human
- 5 umbilical vein endothelial cells, IL-4 induced; Human Thymus Stromal Cells; Soares melanocyte 2NbHM; Soares_placenta_8to9weeks_2NbHP8to9W; Colon Normal III; Hodgkin's Lymphoma II; Normal Human Trabecular Bone Cells; Human Liver; Human endometrial stromal cells-treated with progesterone; Human endometrial stromal cells; HUMAN JURKAT MEMBRANE BOUND POLYSOMES;
- 10 NCI_CGAP_GC4; Human Whole Six Week Old Embryo; normalized infant brain cDNA; Smooth muscle, serum treated; Human Placenta; Human Testes Tumor; Human Synovial Sarcoma; Soares infant brain 1NIB; CD34+ cell, I, frac II; Human Umbilical Vein Endothelial Cells, fract. A; Human Placenta; Human Lung; Stratagene ovary (#937217); Mesangial cell, frac 2; STROMAL -
- 15 OSTEOCLASTOMA; Smooth muscle, IL1b induced; Soares_pregnant_uterus_NbHPU; Human Umbilical Vein, Reexcision; Monocyte activated, re-excision; Human Osteoblasts II; NCI_CGAP_Co2; NCI_CGAP_Co8; NCI_CGAP_Co10; NCI_CGAP_Pr22; Human Umbilical Vein Endothelial Cells, uninduced; Human Pancreas Tumor; T-Cell PHA 24 hrs; Liver, Hepatoma; Human
- 20 Adipose; Ulcerative Colitis; Human Testes Tumor, re-excision; Rejected Kidney, lib 4; Ovarian Tumor 10-3-95; Stratagene liver (#937224); Stratagene corneal stroma (#937222); Fetal Heart; CHME Cell Line, untreated; Soares_NhHMPu_S1; Stratagene HeLa cell s3 937216; NCI_CGAP_Kid5; Soares_parathyroid_tumor_NbHPA; Human Fetal Kidney, Reexcision; Soares_fetal_heart_NbHH19W;
- 25 Soares_senescent_fibroblasts_NbHSF; Activated T-Cell (12hs)/Thiouridine labelledEco; NCI_CGAP_Pan1; Human Primary Breast Cancer Reexcision;

- Monocyte activated; HUMAN B CELL LYMPHOMA; Human Bone Marrow, treated; NCI_CGAP_Ut3; NCI_CGAP_Ov23; NCI_CGAP_Brn23; NCI_CGAP_Brn25; Bone Marrow Cell Line (RS4,II); NCI_CGAP_Ut1; NCI_CGAP_CLL1; NCI_CGAP_Co14; NCI_CGAP_Pr28; Activated T-
- 5 cell(12h)/Thiouridine-re-excision; Human 8 Week Whole Embryo; Nine Week Old Early Stage Human; CD34 positive cells (Cord Blood); PRMIX; Brain, Kozak; Human Osteoarthritic Cartilage Fraction IV; Human Astrocyte; Infant brain, Bento Soares; Soares_testis_NHT; Soares_pineal_gland_N3HPG; Soares_parathyroid_tumor_NbHPA; Soares_fetal_liver_spleen_INFLS_SI;
 - 10 Stratagene hNT neuron (#937233); Sinus piniformis Tumour; Human Osteoarthritic Cartilage Fraction III; Human Normal Cartilage Fraction IV; Tongue Normal; Human Microvascular Endothelial Cells, fract. B; Hypothalamus; Human OB HOS treated (1 nM E2) fraction I; Human OB MG63 control fraction I; Saos2 Cells, Untreated; Human Adult Liver, subtracted; LNCAP untreated; Human Aortic Endothelium;
 - 15 Human Cerebellum, subtracted; Human Primary Breast Cancer, re-excision; Hodgkin's Lymphoma I; Healing Abdomen wound, 70&90 min post incision; Human Pituitary, subtracted; Smooth muscle-ILb induced; H. Epididymus, caput & corpus; NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Human Normal Breast; Human Fetal Epithelium (Skin); Soares_fetal_lung_NbHL19W; H. Kidney Cortex,
 - 20 subtracted; Salivary Gland, Lib 2; Human Osteosarcoma; Human Colon, re-excision; Jurkat T-Cell, S phase; H. Meningioma, M1; Spleen metastatic melanoma; Human Adult Small Intestine; Human Infant Brain; Human Neutrophil; b4HB3MA-Cot18-Bio; b4HB3MA-Cot0.38-HAP-Ft-6; normalized infant brain cDNA; Human Thymus; Human Brain, Striatum; NCI_CGAP_Pr9; NCI_CGAP_Pr10; NCI_CGAP_Pr22;
 - 25 Human Fetal Kidney; NCI_CGAP_AA1; NCI_CGAP_Br2; NCI_CGAP_Br3; NCI_CGAP_Co3; NCI_CGAP_Co9; NCI_CGAP_HN4; NCI_CGAP_Lu1; Human

- Fetal Dura Mater; Human Adult Testes, Large Inserts, Reexcision; Human Heart; Merkel Cells; Soares_testis_NHT; Human Hippocampus; Olfactory epithelium,nasalcavity; Human Placenta (re-excision); Human Activated Monocytes; Human Rhabdomyosarcoma; Soares_NhHMPu_S1; Human Activated T-Cells, re-
- 5 excision; Soares_testis_NHT; Synovial Fibroblasts (control); Hemangiopericytoma; CHME Cell Line,treated 5 hrs; NTERA2, control; normalized infant brain cDNA; Smooth muscle, serum induced,re-exc; Macrophage-oxLDL, re-excision; Resting T-Cell Library,II; 12 Week Old Early Stage Human; Human Eosinophils; NCI_CGAP_Co9; NCI_CGAP_Pr22; NCI_CGAP_Pr25; Colon Carcinoma; Colon
- 10 Normal II; Adipocytes; H Macrophage (GM-CSF treated), re-excision; NCI_CGAP_Br2; Colon Tumor II; Soares_fetal_lung_NbHL19W; Soares_multiple_sclerosis_2NbHMSP; Human Fetal Lung III; 12 Week Early Stage Human II, Reexcision; Human Fetal Heart; Endothelial cells-control; NCI_CGAP_Ut4; NCI_CGAP_Brn23; Soares_fetal_heart_NbHH19W;
- 15 Soares_placenta_8to9weeks_2NbHP8to9W; CD34 depleted Buffy Coat (Cord Blood), re-excision; Human Microvascular Endothelial Cells, fract. A; Smooth muscle,control; HM1; Human retina cDNA randomly primed sublibrary; NCI_CGAP_GC3; Spleen, Chronic lymphocytic leukemia; NCI_CGAP_Co8; NCI_CGAP_GC6; NCI_CGAP_Ut2; NCI_CGAP_CLL1; NCI_CGAP_Kid3;
- 20 NCI_CGAP_Kid5; NCI_CGAP_Kid8; NCI_CGAP_Gas4; NCI_CGAP_Pan1; NCI_CGAP_Brn25; H. Frontal cortex,epileptic,re-excision; Human Endometrial Tumor; Keratinocyte; Soares_pregnant_uterus_NbHPU; Stratagene neuroepithelium (#937231); Human Cerebellum; Soares fetal liver spleen 1NFLS and Stratagene neuroepithelium (#937231).

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 72 as residues: Leu-36 to Ser-49. Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3383 of SEQ ID NO:23, b is an integer of 15 to 3397, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

15 FEATURES OF PROTEIN ENCODED BY GENE NO: 14

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gn|PIDe225817 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "unknown [Schizosaccharomyces pombe]". A partial alignment demonstrating the observed homology is shown immediately below.

25 >gnl|PID|e225817 unknown [Schizosaccharomyces pombe] >pir|S67452|S67452
hypothetical protein SPAC1F12.09 - fission yeast
(Schizosaccharomyces pombe)
Length = 554

Plus Strand HSPs:

Score = 233 (82.0 bits), Expect = 2.1e-17, Sum P(2) = 2.1e-17
Identities = 63/240 (26%), Positives = 107/240 (44%), Frame = +2

5 Q: 1172 LPHVINPVESRLGSSAASLYPVLNFLLYVPELAHSPLYIQDKDGAPVATNAPHSPRWGGI 1351
LP++N E L S P ++F+LYVP PL+++++D + TN+ P+WG I
S: 310 LPNLVNNFEKYLFSFPHIREPTIHFLVLYVPSQIQPLMLENEDSNIIPTNMSLLPQWGS I 369

10 Q: 1352 MVYNVDSKTYNASVLPXXXXXXXFLAQLRLFLGIAQPQLPPKCLLSGPTSEGLM 1531
N +N + L LL GI P LS +
S: 370 TTIN----FNVTEKKLLHDVDLKDYFRVISRDLLLLGINDV---PVSSLSATLA---- 417

15 Q: 1532 TWELDRLLWARSVENXXXXXXXXXXFLGKISNIVIKDDVASEVYKAVAAVQKSAEEL 1711
DRLL R E+ + + + + + + V + + + + + L
S: 418 ----DRLLRQRIAESCIASDTLQNLAKLVHSMQSMAPVKEIQMYVKDTLLSLDMAYKAL 473

20 Q: 1712 ASGHLASAFVASQEAVTSSELAFDPDLLHLLYFPDDQKFAIYIPLFLPMAPVILLSLVK 1891
+ +L A S A + S + A F PS++ +YFPD+ K+ IY PLF P+ +P+L+S +K
S: 474 SQNNLNEALSYSNNAFPSKQEALFHPMSMVTTIYFPDESKEYGIYAPLFAPIILPLISFTK 533

The segment of gnl|PIDe225817 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 123. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities.

- 25 Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

- Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 124 which
30 corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Endometrial Tumor and to a lesser extent in Soares fetal liver spleen INFLS; Soares retina N2b4HR; Human Adult Testes, Large Inserts,
35 Reexcision; Soares melanocyte 2NbHM; Soares_fetal_lung_NbHL19W; Monocyte activated; Soares_total_fetus_Nb2HF8_9w; Soares breast 2NbHBst;

- Soares_fetal_liver_spleen_INFLS_S1; Human Microvascular Endothelial Cells, fract. A; Soares_parathyroid_tumor_NbHPA; T Cell helper I; Activated T-cell(12h)/Thiouridine-re-excision; Soares infant brain 1NIB; Pharynx carcinoma; Human Adult Retina; Human adult (K.Okubo); Stratagene neuroepithelium
- 5 (#937231); Human Tonsils, Lib 2; STROMAL -OSTEOCLASTOMA; Jurkat T-Cell, S phase; Human Infant Brain; normalized infant brain cDNA; Human Umbilical Vein, Reexcision; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; NCI_CGAP_Sch1; Human umbilical vein endothelial cells, IL-4 induced; Human Placenta (re-excision); Synovial Fibroblasts (control); Ovarian Tumor 10-3-95;
- 10 CHME Cell Line,untreated; Human Eosinophils; Smooth muscle, serum treated; Human heart cDNA (YNakamura); Soares_fetal_liver_spleen_INFLS_S1; Human Fetal Lung III; Bone marrow; Human Adult Pulmonary,re-excision; Endothelial cells-control; Human Amygdala; Stratagene pancreas (#937208); Stratagene neuroepithelium (#937231); CD34 positive cells (Cord Blood); Human pancreatic
- 15 islet; Soares_testis_NHT; Human Bone Marrow, treated; Soares placenta Nb2HP and NCI_CGAP_Co12.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 73 as residues: Phe-11 to Gly-16, Pro-33 to Ser-42. Polynucleotides encoding said polypeptides are also provided.

- 20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
- 25 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 2822 of SEQ ID NO:24, b is an integer of 15 to 2836, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 15

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

- 10 gi575681 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "unknown orf, len: 442, CAI: 0.13 [Saccharomyces cerevisiae]". A partial alignment demonstrating the observed homology is shown immediately below.

- 15 >gi|575681 unknown orf, len: 442, CAI: 0.13 [Saccharomyces cerevisiae]
 >pir|S49741|S49741 probable membrane protein YML038c - yeast
 (Saccharomyces cerevisiae)
 Length = 442
- 20 Plus Strand HSPs:
 Score = 142 (50.0 bits), Expect = 9.9e-08, Sum P(2) = 9.9e-08
 Identities = 34/101 (33%), Positives = 57/101 (56%), Frame = +1
- 25 Q: 382 YYCFSIGITFYNNKLT-----SFHFPLFMTMLHLAVIFLFSALSRLVQCSSH---RAR 537
 Y+C SI ++ YN+W+ +P+ +T H A ++L S + L R
 S: 14 YFC-SIALSIYNRMWMPDPKDLGIGYPVLVTFHQA TLWLLSGIYIKLRHKPVKNVLRKN 72
- 30 Q: 538 VVLSWADYLRVRVAPTALATALDVGLSNWSFLYVTVSLYTMK 663
 +W+ +L+ + PTA+A+A D+GLSN SF YV +++YT+ K
 S: 73 NGFNWSFFLKFLPTAVASAGDIGLSNVSFQYVPLTIYTIK 114

The segment of gi575681 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 125. Based on the structural similarity, these homologous

- 35 polypeptides are expected to share at least some biological activities. Such activities

are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

- Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 126 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares retina N2b4HR; Primary Dendritic Cells, lib 1; Soares infant brain 1NIB and to a lesser extent in Soares_total_fetus_Nb2HF8_9w; Activated T-cell(12h)/Thiouridine-re-excision; NCL_CGAP_GCB1; Smooth muscle-ILb induced; Soares adult brain N2b4HB55Y; H. Meningima, M1; Stratagene fetal spleen (#937205); Human Hippocampus; Soares breast 2NbHBst; Human adult testis, large inserts; Colon Tumor II; Human Fetal Kidney, Reexcision; Human Bone Marrow, treated; Keratinocyte; Soares fetal liver spleen 1NFLS; HL-60, unstimulated; Human Tongue, frac 1; H. Striatum Depression, subtracted; Human OB HOS treated (10 nM E2) fraction I; Human Adult Pulmonary; H. Atrophic Endometrium; Breast Lymph node cDNA library; Human Lung; H. Epididymus, cauda; Soares_pineal_gland_N3HPG; Human Umbilical Vein, Endo. remake; NTERA2 + retinoic acid, 14 days; Soares_pregnant_uterus_NbHPU; Human Manic Depression Tissue; Prostate BPH; Human Adult Small Intestine; Human Umbilical Vein, Reexcision; TF-1 Cell Line GM-CSF Treated; Breast Cancer Cell line, angiogenic; Human Brain, Striatum; T-Cell PHA 24 hrs; Soares_testis_NHT; Soares_NFL_T_GBC_S1; Human Pancreas Tumor, Reexcision; Human Activated Monocytes; Epithelial-TNFa and INF induced; Synovial Fibroblasts (control); Human Thymus Stromal Cells; Human Whole Six Week Old Embryo; Human Gall Bladder;

Resting T-Cell Library.II; Smooth muscle, serum treated; Early Stage Human Brain; Human Testes Tumor; Human Synovial Sarcoma; Primary Dendritic cells,frac 2; Human Placenta; human tonsils; Human Fetal Heart; Human Primary Breast Cancer Reexcision; Soares_fetal_heart_NbHH19W; CD34 depleted Buffy Coat (Cord
 5 Blood), re-excision; Colon Normal III; Human Microvascular Endothelial Cells, fract. A; Monocyte activated; HUMAN B CELL LYMPHOMA; T Cell helper I; T cell helper II; Soares placenta Nb2HP; Human retina cDNA Tsp509I-cleaved sublibrary; NCI_CGAP_Co9; NCI_CGAP_GC3; NCI_CGAP_GC4; NCI_CGAP_Co10; NCI_CGAP_Co12; NCI_CGAP_Br1.1 and Soares_fetal_heart_NbHH19W.

10 Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 74 as residues: Pro-67 to Ser-78, Thr-89 to Trp-96, Arg-114 to Ser-125, Ser-147 to Ala-155. Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly
 15 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
 20 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2087 of SEQ ID NO:25, b is an integer of 15 to 2101, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 16

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

- 5 gill1825601 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "weak similarity to rat TEGT protein (GI:456207) [Caenorhabditis elegans]". A partial alignment demonstrating the observed homology is shown immediately below.

10 >gi|1825601 weak similarity to rat TEGT protein (GI:456207)
[Caenorhabditis
elegans] >sp|P91373|P91373 SIMILARITY TO RAT TEGT PROTEIN.
Length = 342

15 Plus Strand HSPs:

Score = 687 (241.8 bits), Expect = 5.9e-67, P = 5.9e-67
Identities = 133/250 (53%), Positives = 180/250 (72%), Frame = +2

20 Q: 440 SNEIGALEKAVIWPQYVKDRIHSTYMYLAGSIGLTALSAIAISRTPLVMFMHGRGWSVTI 619
S E ++K+ IWP YV++RI +TY YLAGS+ LTA+S +A SR+ +M G +++
S: 93 SKEQSILQKSAIWPSYVRERISTTYAYLAGSLALTAVSGVAASRSAAIMRLTAGGMMSL 152

Q: 620 GVTFAMVVGAGMLVRSIPYDQSPGPKHLAWLLHSGVMGAVVAPLTILGPELLIRAAWYTA 799
T AAN+ +GML RSI Y+ + KHLAW LH GV+GAV APL + GP+L RAAWYTA
25 S: 153 FTWAAMIASGMLARSIDYESTVA-KHLAWALHCGVLGAVFAPLCFMAGVLTAAWYTA 211

Q: 800 GIVGGLSTVAMCAPSEKFLNMGAPLGVGLGVFVSSLSGSMFLPTTVAGATLYSVANYGG 979
GIVGGLS A+ APSEKFL M PL +G G+VFV+++G+ FLPP + GA+L S+ +YGG
30 S: 212 GIVGGLSATAITAPSEKFLMMSGPLAMGFGVVPVANIGAFFLPPGSGALGASLAIIVYGG 271

Q: 980 LVLFSMFLLYDTQKVIKRAEVSP----MYG----VQKYDPINSMLSIYMDTLNIFMNRVAT 1135
L+LFS FLLYDTQ+++K+AE P +YG ++ +DPIN+ +SIYMD LNIFMNR+
S: 272 LILFSAFLLYDTQRLVKKAENHPHSSQLYGSMDQIRSFDPINAQMSIYMDVLNIFMRLVM 331

35 Q: 1136 MLA-TGGNRRKK 1165
++ GNR+K
S: 332 IMGGMNGNRRKK 342

- The segment of gill1825601 that is shown as "S" above is set out in the
- 40 sequence listing as SEQ ID NO. 127. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein.

Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

- Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 128 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain 1NIB and to a lesser extent in Primary Dendritic Cells, lib 1; Keratinocyte; Monocyte activated; Human Testes; Normal colon; T cell helper II; Human Heart; Macrophage (GM-CSF treated); Soares fetal liver spleen 1NFLS; Human Amygdala, re-excision; Human Osteoclastoma, re-excision; KMH2; Adipocytes; Human fetal heart, Lambda ZAP Express; Human retina cDNA Tsp509I-cleaved sublibrary; 12 Week Early Stage Human II, Reexcision; Nine Week Old Early Stage Human; Human Cerebellum;
- 15 NCI_CGAP_Pr2; Human Colon Cancer, subtracted; Soares retina N2b4HR; Human Osteoclastoma Stromal Cells - unamplified; Human Infant Brain; human ovarian cancer; normalized infant brain cDNA; Human Adrenal Gland Tumor; Fetal Liver, subtraction II; Human Gall Bladder; NCI_CGAP_Kid5; Soares melanocyte 2NbHM; Human Testes, Reexcision; Soares_senescent_fibroblasts_NbHSF; Human colon carcinoma (HCC) cell line, remake; Human Adult Pulmonary; Human Adult Heart, re-excision; Soares_pregnant_uterus_NbHPU; Jurkat T-Cell, S phase; Human Manic Depression Tissue; STRATAGENE Human skeletal muscle cDNA library, cat. #936215; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Stromal cell TF274; Soares_fetal_liver_spleen_1NFLS_S1; Hepatocellular Tumor, re-excision;
- 25 Smooth muscle, serum induced, re-exc; Macrophage-oxLDL, re-excision; Human Adult Pulmonary, re-excision; Endothelial cells-control; Human Osteoclastoma;

- Human Amygdala; Bone Marrow Cell Line (RS4,11); NCI_CGAP_Pan1;
 NCI_CGAP_Lym12; Soares placenta Nb2HP; Testis, normal; Prostate; HPAS
 (human pancreas, subtracted); Fetal Heart, re-excision; Human Liver; Stratagene
 ovary (#937217); Stratagene schizo brain S11; Human Whole Brain #2 - Oligo dT >
 5 1.5Kb; Human endometrial stromal cells; Stratagene colon (#937204); Myeloid
 Progenitor Cell Line; Stratagene muscle 937209; Stratagene endothelial cell 937223;
 Brain Frontal Cortex, re-excision; Human Adult Small Intestine; T-Cell PHA 16 hrs;
 Soares_NhHMPu_S1; Stratagene HeLa cell s3 937216;
 Soares_fetal_liver_spleen_1NFLS_S1; NCI_CGAP_Brn25; Human Thymus; L428;
 10 NCI_CGAP_GCB1; Human Umbilical Vein Endothelial Cells, uninduced;
 Macrophage-oxLDL; Human Hypothalamus, Schizophrenia; Human Placenta (re-
 excision); Human Thymus Stromal Cells; Human Whole Six Week Old Embryo;
 NCI_CGAP_Kid5; Ovarian Tumor 10-3-95; PC3 Prostate cell line; Colon Tumor;
 Human T-Cell Lymphoma; Smooth muscle, serum treated; Human Placenta;
 15 Stratagene hNT neuron (#937233); NCI_CGAP_Kid3; Stratagene neuroepithelium
 (#937231); Human Fetal Lung III; human tonsils; Activated T-Cell
 (12hs)/Thiouridine labelled Eco; Soares_fetal_liver_spleen_1NFLS_S1; Human
 Microvascular Endothelial Cells, fract. A; Spleen, Chronic lymphocytic leukemia; T
 Cell helper 1; NCI_CGAP_Brn25; Activated T-cell(12h)/Thiouridine-re-excision;
 20 Soares_pineal_gland_N3HPG; Stratagene ovarian cancer (#937219);
 Soares_fetal_heart_NbHH19W; Human 8 Week Whole Embryo; NCI_CGAP_Co8;
 NCI_CGAP_Ut3; Stratagene muscle 937209; Stratagene colon (#937204); Stratagene
 neuroepithelium NT2RAMI 937234; Human Pineal Gland; H.Leukocytes, normalized
 cot 5B; H. Leukocytes, normalized cot 5A; Kidney Cortex; Healing Abdomen
 25 Wound, 15 days post incision; CD34+cells, II; Thyroid Thyroiditis; Human Infant
 Adrenal Gland, Subtracted; Human Fetal Heart, Differential (Fetal-Specific); Thyroid

- Tumour; Normal Prostate; Human Adult Heart; K562 + PMA (36 hrs); Human Normal Cartilage, Fraction I; Human Normal Cartilage Fraction II; NCI_CGAP_GCB1; Human osteoarthritis, fraction I; Human Prostate, subtracted; Colorectal Tumor; NCI_CGAP_Co3; Saos2, Dexamethosome Treated; Human OB
- 5 HOS treated (1 nM E2) fraction I; LNCAP untreated; Frontal Lobe, Dementia; Human Colon; Human (HCC) cell line liver (mouse) metastasis, remake; Human Colon Carcinoma (HCC) cell line; Hodgkin's Lymphoma I; Human Pituitary, subtracted; Human Neutrophils, Activated, re-excision; Aorta endothelial cells + TNF- α ; Activated T-cells; Human Thyroid; Human Quadriceps; Human Soleus;
- 10 Smooth Muscle- HASTE normalized; Cem cells cyclohexamide treated; NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Raji Cells, cyclohexamide treated; Human adult small intestine, re-excision; Human Lung Cancer, re-excision; Pancreas Tumor PCA4 Tu; Human Hypothalamus, schizophrenia, re-excision; Human endometrial stromal cells-treated with estradiol; Infant brain, Bento Soares;
- 15 STRATAGENE Human skeletal muscle cDNA library, cat. #936215.; Soares_testis_NHT; Soares_parathyroid_tumor_NbHPA; Hepatocellular Tumor, re-excision; Hepatocellular Tumor; pBMC stimulated w/ poly I/C; Alzheimers, sponge change; NTERA2 + retinoic acid, 14 days; H. Kidney Cortex, subtracted; Human Stomach, re-excision; LNCAP prostate cell line; Human Osteosarcoma; Healing groin
- 20 wound, 6.5 hours post incision; H. Lymph node breast Cancer; Human Neutrophil; Human Prostate; Gessler Wilms tumor; HTC DL1; Jia bone marrow stroma; Liver HepG2 cell line.; Soares_testis_NHT; Soares_pregnant_uterus_NbHPU; Stratagene hNT neuron (#937233); Mo7e Cell Line GM-CSF treated (1ng/ml); Cot250Ft-b4HB3MA; Human fetal lung; Human pancreatic islet; Normalized infant brain,
- 25 Bento Soares; Soares_parathyroid_tumor_NbHPA; TF-1 Cell Line GM-CSF Treated; Breast Cancer Cell line, angiogenic; NCI_CGAP_Kid3; NCI_CGAP_AA1;

- NCI_CGAP_Co3; NCI_CGAP_Co9; NCI_CGAP_Ew1; NCI_CGAP_GC4;
 NCI_CGAP_Lu1; NCI_CGAP_Pr2; NCI_CGAP_Co10; NCI_CGAP_Pr22; Human
 Uterine Cancer; Human Activated T-Cells; Human Adult Testes, Large Inserts,
 Reexcision; T-Cell PHA 24 hrs; Human Hippocampus;
- 5 Soares_fetal_lung_NbHL19W; Soares_multiple_sclerosis_2NbHMSP; Human
 umbilical vein endothelial cells, IL-4 induced; Human Activated Monocytes; Spinal
 cord; STRATAGENE Human skeletal muscle cDNA library, cat. #936215; Human
 Activated T-Cells, re-excision; Human Chondrosarcoma; Ulcerative Colitis; Synovial
 Fibroblasts (control); Soares breast 2NbHBst; CHME Cell Line, treated 5 hrs;
- 10 Soares_testis_NHT; Soares_fetal_heart_NbHH19W; NCI_CGAP_Co14;
 NCI_CGAP_Brn23; NTERA2, control; Stratagene lung (#937210); Human Liver,
 normal; Soares_testis_NHT; Fetal Heart; Human pancreatic islet; Human fetal heart,
 Lambda ZAP Express; CHME Cell Line, untreated; Human Ovarian Cancer
 Reexcision; Colon Normal II; H Macrophage (GM-CSF treated), re-excision; Human
- 15 Testes Tumor; Dendritic cells, pooled; NCI_CGAP_GC4; NCI_CGAP_Lu5;
 NCI_CGAP_Ov2; NCI_CGAP_Co12; NCI_CGAP_GCB1; NCI_CGAP_Pr25;
 NCI_CGAP_Brn25; Primary Dendritic cells, frac 2; Human Fetal Heart; Endothelial-
 induced; Colon Normal III; NCI_CGAP_Kid1; Anergic T-cell; Smooth
 muscle, control; Soares_fetal_heart_NbHH19W; Human Bone Marrow, treated;
- 20 Soares_fetal_lung_NbHL19W; NCI_CGAP_Ut1; NCI_CGAP_Lu24;
 Soares_fetal_lung_NbHL19W; Soares_fetal_heart_NbHH19W;
 Soares_pregnant_uterus_NbHPU; H. Frontal cortex, epileptic, re-excision; Hodgkin's
 Lymphoma II; NCI_CGAP_Lu5; NCI_CGAP_Ut4; NCI_CGAP_Gas4;
 NCI_CGAP_Kid5; NCI_CGAP_Lym5 and Stratagene HeLa cell s3 937216.
- 25 Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2032 of SEQ ID NO:26, b is an integer of 15 to 2046, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 17

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gnl|PIDe214699 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "ARF-like protein 5 [Rattus norvegicus]." This rat protein is thought to be a novel member of the ARF-family of Ras-related GTPases. A partial alignment demonstrating the observed homology is shown immediately below.

```
>gnl|PID|e214699 ARF-like protein 5 [Rattus norvegicus] >pir|S72161|S72161
ADP-ribosylation factor 5 - rat >sp|P51646|ARL5_RAT
ADP-RIBOSYLATION FACTOR-LIKE PROTEIN 5.
Length = 179
```

Plus Strand HSPs:

```
Score = 794 (279.5 bits), Expect = 3.5e-78, P = 3.5e-78
Identities = 144/179 (80%), Positives = 165/179 (92%), Frame = +3
```

```
Q: 192 NGLIFAKLMSLFCNQEHKVIIVGLDNAGKTTILYQFLMNEVVHTSPTIGSNVEEIVVKNT
MG++F ++W LF +QEHEVIIIVGLDNAGKTTILYQF MNEVVHTSPTIGSNVEEIVV NT
S: 1 MGILETRIWRFLFNHQBKVIIVGLDNAGKTTILYQFSMNEVVHTSPTIGSNVEEIVVNNT 60
```


Q: 372 HFLMWDIGGQESLRSSWNTYYSNTEFIIILVVDSIDRERLATTKEELYRMLAHEDLRKAAV 551
 FLMWDIGGQESLRSSWNTYY+NTEF+I+VVDS DRER+++T+EELY+MLAHEDLRKA +
 S: 61 RFLMWDIGGQESLRSSWNTYYTNTFVIVVDSTDREIRISVTREELYKMLAHEDLRKAGL 120

5 Q: 552 LIFANKQDMKGCMTAAEISKYLTLSSTKDPWHIQSCCALTGEGLCQGLEWMTSRIGVR 728
 LIFANKQD+K CMT AETS++L L+STKDH WHIQ+CCALTGEGLCQGLEWM SR+ +R
 S: 121 LIFANKQDVKECHTVAETSQFLKLTSTKDHQWHIQACCALTGEGLCQGLEWMSRLKIR 179

The segment of gnlIPIDie214699 that is shown as "S" above is set out in the
 10 sequence listing as SEQ ID NO. 129. Based on the structural similarity, these
 homologous polypeptides are expected to share at least some biological activities.
 Such activities are known in the art, some of which are described elsewhere herein.
 Assays for determining such activities are also known in the art, some of which have
 been described elsewhere herein.

15 Preferred polypeptides of the invention comprise a polypeptide having the
 amino acid sequence set out in the sequence listing as SEQ ID NO. 130 which
 corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a
 sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following
 20 tissues/cDNA libraries: Nine Week Old Early Stage Human;
 Soares_NFL_T_GBC_S1 and to a lesser extent in Activated T-Cells, 12hrs,
 differentially expressed; Hodgkin's Lymphoma I; Soares_testis_NHT; Human T-cell
 lymphoma, re-excision; Pancreas Tumor PCA4 Tu; pBMC stimulated w/ poly I/C;
 Human Umbilical Vein, Reexcision; KMH2; Soares_multiple_sclerosis_2NbHMSp;
 25 Human Activated T-Cells; Liver, Hepatoma; Human Thymus Stromal Cells;
 Hepatocellular Tumor, re-excision; Human Ovarian Cancer Reexcision; Bone
 Marrow Cell Line (RS4,11); Hodgkin's Lymphoma II; Human 8 Week Whole
 Embryo; Soares_NhHMPu_S1 and Primary Dendritic Cells, lib 1.

Given the homology of this protein to the ras-related ARF-family of GTPase rat proteins, the expression of this protein in vivo where the ras proto-oncogene has undergone a mutation to the oncogene may reduce or eliminate the activity of the ras protein. When the ras proto-oncogene undergoes a mutation to the oncogene, the activity of the associated GTPases is greatly reduced. Thus, expression of this protein in vivo may reduce or terminate the activity of the ras oncogene. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3555 of SEQ ID NO:27, b is an integer of 15 to 3569, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 18

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gnllPIDd1021323 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "peptide/histidine

25

transporter [Rattus norvegicus].* This rat peptide/histidine transporter has been shown to be expressed in the brain and retina, and is thought to be involved in histidine transport. A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >gnl|PID|d1021323 (AB000280) peptide/histidine transporter (Rattus
      norvegicus)>sp|O09014|O09014 PEPTIDE/HISTIDINE TRANSPORTER.
      Length = 572

10      Plus Strand HSPs:

      Score = 1538 (541.4 bits), Expect = 1.3e-159, Sum P(2) = 1.3e-159
      Identities = 303/385 (78%), Positives = 324/385 (84%), Frame = +3

15      Q: 330 RRFNFNWFYSINLGAILSLGGIAYIQQNVSFVTGVAIPTVCVGLAFVXFLCGQSVFITKP 509
      RRFNFNWFYSINLGAILSLGGIAYIQQNVSF+TGY IPTVCV +AF+ FLCGQSVFITKP
      S: 193 RRFNFNWFYSINLGAILSLGGIAYIQQNVSF+TGYLTIPTVCVAIAFLVFLCGQSVFITKP 252

20      Q: 510 PDGSAFTDMFKILTYSCCSQKRSGERQSNBEGIGVFQSSKQSLDFDSCMKSHGGPFTEEK 689
      PDGSAFTDMF+ILTYSCCSQ R G+R+S GEG+GVFQSSK SLDFDSCMS GGPFE+K
      S: 253 PDGSAFTDMFRILTYSCCSQ-RGGQRRS-GEGLGVFQSSKSLDFDSCMSRGGPFTEDEK 310

25      Q: 690 VEDVKALVKIVPVFLALIPYWTVYFQMOTTYVLQSLHLRIPEISNITTTHTLPAAWLTH 869
      VEDVKALVKIVPVFLALIPYWTVYFQMOTTYVLQSLHL+IPEIS+ITTT HTLPAAWLTH
      S: 311 VEDVKALVKIVPVFLALIPYWTVYFQMOTTYVLQSLHLRIPEISSITTTHTLPAAWLTH 370

30      Q: 870 FDAXXXXXXXXXXXXXXXXXXILRRHGLLPSSLKRIAVGMFFVMCSAFAAGILESKRLNLVK 1049
      FDA +LRRHGLLPSSLKRIAVGMFFV CSAFAAGILESKRL+LVK
      S: 371 FDAVLILLILLIPLDKLVDVPLRRHGLLPSSLKRIAVGMFFVTCSAFAAGILESKRLDLVK 430

35      Q: 1050 EKTINQITIGNVVYHAADLSLWQVPQYLLIGISEIFASITAGLEFAYSAAPKSMQSAIMXX 1229
      EKTINQITIG VVYHAADL +WNQ+PQY+LIGISEIFASITAGLEFAYSAAPKSMQSAIM
      S: 431 EKTINQITIGVVYHAADLPWNQIPQYVLIGISEIFASITAGLEFAYSAAPKSMQSAIMGL 490

40      Q: 1230 XXXXXXXXXXXXXXXXXXXXKAIGWMSSTDFGHNINGCYLNYYFFLLAAIQGATLLFL 1409
      +KAIGWMSSTDFGHNIN C+L+YFFLLAAIQGATLLFL
      S: 491 FFFFSGIGSVGSGLLALVSLKAIGWMSSTDFGHNINCHLHYFFLLAAIQGATLLFL 550

      Q: 1410 IISVKYDHRDHQRSRANGVPTSR 1484
      I+SVKYD QR+R +G S R
      S: 551 IISVKYDR----QRATDGGTASTR 571
  
```

The segment of gnl|PID|d1021323 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 131. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein.

Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 132 which
 5 corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Osteoclastoma and to a lesser extent in Human Endometrial Tumor; NCI_CGAP_GCB1; Soares_pregnant_uterus_NbHPU; Soares
 10 fetal liver spleen INFLS; Morton Fetal Cochlea; Hepatocellular Tumor; Stratagene ovarian cancer (#937219); Soares_fetal_heart_NbHH19W; Hemangiopericytoma; CHME Cell Line, treated 5 hrs; Smooth muscle, serum induced, re-exc; NCI_CGAP_Lu5; Soares_NhHMPu_S1; Soares_testis_NHT; Soares_pineal_gland_N3HPG; Stratagene lung carcinoma 937218; Activated T-
 15 cell(12h)/Thiouridine-re-excision; Soares infant brain INIB; Spleen/normal; Human Fetal Brain; Human colon carcinoma (HCC) cell line, remake; Human Pancreatic Carcinoma; HUMAN STOMACH; Pancreatic Islet; Smooth Muscle Serum Treated, Norm; Human Thyroid; Human Tonsils, Lib 2; Apoptotic T-cell, re-excision; B Cell lymphoma; Morton Fetal; Human Chronic Synovitis; T-Cell PHA 16 hrs; Human
 20 Fetal Dura Mater; Human Adult Testes, Large Inserts, Reexcision; Soares_NhHMPu_S1; Stratagene HeLa cell s3 937216; Merkel Cells; Pancreatic Islet; Soares_senescent_fibroblasts_NbHSF; Human Ovary; Human Activated Monocytes; Soares_fetal_liver_spleen_INFLS_S1; Human adult testis, large inserts; Stratagene lung (#937210); Macrophage-oxLDL, re-excision; Resting T-Cell
 25 Library, II; Colon Normal II; Adipocytes; Dendritic cells, pooled; Human Fetal Lung III; Human pancreatic islet; NCI_CGAP_Co3; NCI_CGAP_GC4; NCI_CGAP_Kid6;

Soares_NFL_T_GBC_S1; Soares_fetal_heart_NbHH19W;
Soares_pregnant_uterus_NbHPU; Soares_fetal_liver_spleen_1NFLS_S1; b4HB3MA
Cot8-HAP-Ft and Primary Dendritic Cells, lib 1.

The tissue distribution in neural tissues, in conjunction with the homology to
5 the histidine transporter expressed in the brain, suggests that the protein product of
this clone is useful for the detection/treatment of neurodegenerative disease states and
behavioural disorders such as Alzheimers Disease, Parkinsons Disease, Huntingtons
Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive
compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism,
10 and altered behaviors, including disorders in feeding, sleep patterns, balance, and
perception. In addition, the gene or gene product may also play a role in the treatment
and/or detection of developmental disorders associated with the developing embryo,
or sexually-linked disorders. Protein, as well as, antibodies directed against the
protein may show utility as a tumor marker and/or immunotherapy targets for the
15 above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly
available and accessible through sequence databases. Some of these sequences are
related to SEQ ID NO:28 and may have been publicly available prior to conception of
the present invention. Preferably, such related polynucleotides are specifically
20 excluded from the scope of the present invention. To list every related sequence
would be cumbersome. Accordingly, preferably excluded from the present invention
are one or more polynucleotides comprising a nucleotide sequence described by the
general formula of a-b, where a is any integer between 1 to 2542 of SEQ ID NO:28, b
is an integer of 15 to 2556, where both a and b correspond to the positions of
25 nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a
+ 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 19

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil1872200 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "alternatively spliced product using exon 13A [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

10  >gi|1872200 alternatively spliced product using exon 13A [Homo sapiens]
    >sp|P78525|P78525 MYB PROTO-ONCOGENE PROTEIN (C-MYB).
    Length = 666

15  Minus Strand HSPs:

    Score = 237 (83.4 bits), Expect = 6.4e-17, P = 6.4e-17
    Identities = 55/94 (58%), Positives = 60/94 (63%), Frame = -1

20  Q:   831 LSLSLFEMESCLV-A*AGVQWHDLSL*PPPLGFK*FSCLSLPNRNDYKVTCTKPGQXXX 655
      L+  LF  S +  A  GVQWHD GSL P P GFK FSCLSLP  NDY+   +P
      S:   551 LNTQLFTQTSPVADAPTVGVQWHDGSLQLPLPPGFKRFSCLSLPRSDYRHPPPRANFEF 610

25  Q:   654 XXXLVEMGFHHIGQAGLELLTSNDRSASASQSAGI 550
      LVE GF H+GQAGLELLTS D ASASQSA I
      S:   611 ---LVETGFLHVGQAGLELLTSGDLPASASQSARI 642
  
```

The segment of gil1872200 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 133. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 134 which

corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: normalized infant brain cDNA; NCI_CGAP_GCB1 and to a
- 5 lesser extent in Activated T-cell(12h)/Thiouridine-re-excision; Keratinocyte; Human Cerebellum; Soares fetal liver spleen 1NFLS; H Macrophage (GM-CSF treated), re-excision; Soares melanocyte 2NbHM; Nine Week Old Early Stage Human; Raji cells, cyclohexamide treated, subtracted; Saos2 Cells, Untreated; Smooth muscle, IL1b induced; CHME Cell Line, treated 5 hrs; Stratagene HeLa cell s3 937216; Fetal Liver,
 - 10 subtraction II; Ovarian Tumor 10-3-95; Adipocytes; Soares_total_fetus_Nb2HF8_9w; Activated T-Cell (12hs)/Thiouridine labelledEco; NCI_CGAP_Co3; Osteoblasts; Human 8 Week Whole Embryo; Prostate; H.Leukocytes, normalized cot 50A3; Human Umbilical Vein Endothelial Cells, fract. B; Soares_NhHMPu_S1; Soares ovary tumor NbHOT; Normal Prostate; Tongue Tumour; Bone Cancer; H. Frontal
 - 15 Cortex, Epileptic; Supt Cells, cyclohexamide treated; Smooth muscle, control, re-excision; Smooth muscle-ILb induced; Aorta endothelial cells + TNF-a; H. cerebellum, Enzyme subtracted; Amniotic Cells - TNF induced; Soares retina N2b4HR; Cem cells cyclohexamide treated; Apoptotic T-cell, re-excision; B Cell lymphoma; STROMAL -OSTEOCLASTOMA; Hepatocellular Tumor; Human
 - 20 endometrial stromal cells; Synovial Fibroblasts (II1/TNF), subt; Human Prostate; Human Bone Marrow, re-excision; Soares_total_fetus_Nb2HF8_9w; KMH2; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Human Fetal Dura Mater; Human Placenta (re-excision); Epithelial-TNFa and INF induced; Human fetal heart, Lambda ZAP Express; Stratagene fibroblast (#937212); Human Testes Tumor,
 - 25 re-excision; Bone Marrow Stromal Cell, untreated; Human Fetal Brain; Stratagene ovarian cancer (#937219); Macrophage (GM-CSF treated);

- Soares_total_fetus_Nb2HF8_9w; Soares_parathyroid_tumor_NbHPA;
 Soares_senescent_fibroblasts_NbHSF; Stratagene lung (#937210); Smooth muscle,
 serum induced, re-exc; Macrophage-oxLDL, re-excision; Soares_NhHMPu_S1; PC3
 Prostate cell line; Human T-Cell Lymphoma; Human Fetal Kidney, Reexcision;
 5 Human Synovial Sarcoma; Human Fetal Lung III; Monocyte activated; Neutrophils
 IL-1 and LPS induced; Bone Marrow Cell Line (RS4,11); NCI_CGAP_Br2;
 NCI_CGAP_Co9; NCI_CGAP_GC4; NCI_CGAP_Kid3; NCI_CGAP_Kid5;
 Hodgkin's Lymphoma II; Soares_NhHMPu_S1; Stratagene NT2 neuronal precursor
 937230; T cell helper II; Soares placenta Nb2HP; Primary Dendritic Cells, lib 1 and
 10 Soares infant brain INIB.

Preferred polypeptides of the present invention comprise immunogenic
 epitopes shown in SEQ ID NO: 78 as residues: Thr-28 to Leu-34, Pro-36 to Trp-47.
 Polynucleotides encoding said polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly
 15 available and accessible through sequence databases. Some of these sequences are
 related to SEQ ID NO:29 and may have been publicly available prior to conception of
 the present invention. Preferably, such related polynucleotides are specifically
 excluded from the scope of the present invention. To list every related sequence
 would be cumbersome. Accordingly, preferably excluded from the present invention
 20 are one or more polynucleotides comprising a nucleotide sequence described by the
 general formula of a-b, where a is any integer between 1 to 1135 of SEQ ID NO:29, b
 is an integer of 15 to 1149, where both a and b correspond to the positions of
 nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a
 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 20

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: NCI_CGAP_GCB1; Soares_testis_NHT and to a lesser extent in Soares_fetal_liver_spleen_1NFLS_S1; Soares placenta Nb2HP; Soares fetal liver spleen 1NFLS; Human Adult Pulmonary; NCI_CGAP_Alv1; Human Colon, re-excision; Spleen metastatic melanoma; Spinal Cord, re-excision; Bone Marrow Stromal Cell, untreated; Human Ovarian Cancer Reexcision; Human Osteoclastoma and Soares_parathyroid_tumor_NbHPA.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1853 of SEQ ID NO:30, b is an integer of 15 to 1867, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 21

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. pirJCS238IJC5238 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "galactosylceramide-

25

like protein, GCP - human". A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >pir|JC5238|JC5238 galactosylceramide-like protein, GCP - human
      Length = 114

      Minus Strand HSPs:

10     Score = 148 (52.1 bits), Expect = 3.9e-09, P = 3.9e-09
      Identities = 32/50 (64%), Positives = 36/50 (72%), Frame = -1

      Q:  927 KAGSGGSCLSRHLGRPRWVDHLRSGVRDQPDQYGETPISTKNTKISSGG 778
           +AG G          LGRPR VDHLRSGVRDQP Q+G+TP ST+NTKIS  G
15     S:  62 QAGHGALTCNPSTLGRPRQVDHLRSGVRDQPGHGKTP-STRNTKISGRG 110

```

The segment of pir|JC5238|JC5238 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 135. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein.

20 Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 136 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a

25 sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: wilm's tumor and to a lesser extent in Soares ovary tumor NbHOT; NCI_CGAP_GC4; Soares_NhHMPu_S1; Infant brain, Bento Soares; Soares_total_fetus_Nb2HF8_9w; Stratagene hNT neuron (#937233); Human Tongue,

30 frac 2; Soares retina N2b4HR; Alzheimers, spongy change; NCI_CGAP_Co3; NCI_CGAP_Kid3; Soares_pregnant_uterus_NbHPU; Stratagene fetal retina 937202;

Spinal Cord, re-excision; Human Activated T-Cells, re-excision; Smooth muscle, control and Soares fetal liver spleen INFLS.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 80 as residues: Arg-84 to Cys-90, Gln-138 to Leu-

- 5 155. Polynucleotides encoding said polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
- 10 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1258 of SEQ ID NO:31, b is an integer of 15 to 1272, where both a and b correspond to the positions of
- 15 nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 22

- The computer algorithm BLASTX has been used to determine that the
- 20 translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi500832 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "Yhr074wp [Saccharomyces cerevisiae]". A partial alignment demonstrating the observed
- 25 homology is shown immediately below.

>gi|500832 Yhr074wp [Saccharomyces cerevisiae] >pir|S46811|S46811
hypothetical

protein YHR074w - yeast (Saccharomyces cerevisiae)
Length = 714

5

Plus Strand HSPs:

Score = 913 (321.4 bits), Expect = 6.5e-91, P = 6.5e-91
Identities = 179/325 (55%), Positives = 226/325 (69%), Frame = +1

10

Q: 1036 RSGNEEVADVRTIVNQ-ISYXPQDPRDLGRLTTCYMASKNSSQETCTRARELAQQIG 1212
++GNE+V+ DVR I + P P+DL +I +C+M ++HSS+ET RA++L+ IG
S: 385 QNGNEQVIKDVRKITRSGDDWIPDSQDLASKIFHSCFMGTENSSKETRNRAKDLNSAIG 444

15

Q: 1213 SHHISLNIDPAVKAVMGIFSLVTGKSPLFAAHGSSRENALQNVQARIMVLAYLFAQL 1392
S+H+ L +D V +V+ +F + TKG P++ GGS ENLALQN+QAR+RMVL+YLFPAQL
S: 445 SYHVDLKMDSLVS SVSLFEVATGKKPIYKIFGGSQIENLALQNIQARLRMVL SYLFAQL 504

20

Q: 1393 SLWSRGV--HGGLLVLGSANVDESLLGYLTKYDCSSADINPIGGISKTDLRAFPQCIQR 1566
W RG+ GGLLVLGSANVDE L GYLTKYDCSSADINPIGGISKTDL+ F+ + ++
S: 505 LPWVRGIPNSGGLLVLGSANVDECLRGYLTKYDCSSADINPIGGISKTDLKRFIAYASKQ 564

25

Q: 1567 FQLPALQSIXXXXXXXXXXXXXGGVQSQTDEEDMGMTYAEISVYGKLRKRVAKMGYPYSMF 1746
+ P L V Q+DE DMGMTY EL V+G LRRV K GPYSMF
S: 565 YNMPILNDFLNATPTAELEPMTKD YV-QSDEIDMGMTYEEELGVGYLRKRVKEKGPYSMFL 623

30

Q: 1747 KLLGMWRHICTPRQVADKVKRRFFSKYSMMNRHKMTTLTPAYHAENYSPEDNRFDLRPFLLYN 1926
KLL W TPRQ+++KVKRRFF Y++NRHK T LTP+YHAE YSPEDNRFDLRPFLL N
S: 624 KLLHQSFKLTPRQISEKVRFFFFFYAINRHKQTVLTPSYHAEQYSPEDNRFDLRPFLLIN 683

Q: 1927 TSWPWFQFCIENQVLQLERAEPQSLD 2004
+PW R I+ V Q E + +LD
S: 684 PRFPWASRKIDEVVEQCEAHKGSTLD 709

35

The segment of gi|500832 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 137. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

40

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 138 which

corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares_total_fetus_Nb2HF8_9w and to a lesser extent in
- 5 NCI_CGAP_Co3; Stratagene muscle 937209; Soares_NhHMPu_S1; breast lymph node cDNA library; HUMAN B CELL LYMPHOMA; Human Eosinophils; Larynx Tumour; Human Lung; Mesangial cell, frac 2; Breast Cancer cell line, MDA 36; Human Adult Small Intestine; Human Neutrophil; Soares_parathyroid_tumor_NbHPA; H. Kidney Medulla, re-excision; Human
- 10 Pancreas Tumor; NCI_CGAP_Co8; NCI_CGAP_Co12; Human Adult Testes, Large Inserts, Reexcision; Macrophage-oxLDL; Macrophage (GM-CSF treated); Human adult testis, large inserts; Macrophage-oxLDL, re-excision; Barstead spleen HPLRB2; NCI_CGAP_Co8; Soares_testis_NHT; Soares_NFL_T_GBC_S1; Colon Tumor; Smooth muscle, serum treated; Human Placenta; Colon Tumor II; Primary Dendritic
- 15 cells, frac 2; Human Fetal Heart; Endothelial cells-control; Human Primary Breast Cancer Reexcision; Human Microvascular Endothelial Cells, fract. A; Smooth muscle, control; Human Bone Marrow, treated; Spleen, Chronic lymphocytic leukemia; T Cell helper I and Hodgkin's Lymphoma II.

- Preferred polypeptides of the present invention comprise immunogenic
- 20 epitopes shown in SEQ ID NO: 81 as residues: Arg-36 to Ala-43. Polynucleotides encoding said polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of
- 25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2221 of SEQ ID NO:32, b is an integer of 15 to 2235, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 23

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi|531829 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "beta-galactosidase alpha peptide [Cloning vector pSport1]". A partial alignment demonstrating the observed homology is shown immediately below.

```

>gi|531829 beta-galactosidase alpha peptide [Cloning vector pSport1]
>sp|Q46478|Q46478 BETA-GALACTOSIDASE ALPHA PEPTIDE (FRAGMENT).
{SUB
  1-113}
  Length = 114

  Plus Strand HSPs:

  Score = 96 (33.8 bits), Expect = 8.9e-10, Sum P(2) = 8.9e-10
  Identities = 17/20 (85%), Positives = 17/20 (85%), Frame = +1

  Q:      4 TLYKECWYACRYRSGIPGST 63
          T Y E WYACRYRSGIPGST
  S:      11 THYRESWYACRYRSGIPGST 30

  Score = 85 (29.9 bits), Expect = 8.9e-10, Sum P(2) = 8.9e-10
  Identities = 15/19 (78%), Positives = 17/19 (89%), Frame = +2

  Q:      1901 GGRSRGSKLTYACIRHHXA 1957
           GGRSRGSKLTYAC+R H +
  S:      35 GGRSRGSKLTYACMRHHSS 53

```

The segments of gil531829 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 139 and SEQ ID NO. 141. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 140 and/or SEQ ID NO. 142 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares fetal liver spleen 1NFLS and to a lesser extent in Soares infant brain 1NIB; Stratagene HeLa cell s3 937216; Human Fetal Heart; Soares_fetal_heart_NbHH19W; NCI_CGAP_Lu5; Synovial Fibroblasts (control); NCI_CGAP_Brn25; Stratagene lung (#937210); Soares_NhHMPu_S1; Human retina cDNA randomly primed sublibrary; Human Testes; Human Endometrial Tumor; Soares_pregnant_uterus_NbHPU; Osteoblasts; Soares placenta Nb2HP; Human Gastrocnemius; Human Leukocytes; Human Cardiomyopathy, subtracted; Human Adult Retina; Activated T-cells; Stratagene colon (#937204); HEL cell line; Alzheimers, spongy change; Salivary Gland, Lib 2; Human Colon, re-excision; Human Infant Brain; T-Cell PHA 16 hrs; Human Brain, Striatum; NCI_CGAP_Br3; NCI_CGAP_Co3; NCI_CGAP_Pr1; NCI_CGAP_GCB1; Monocyte activated, re-excision; Human Uterine Cancer; Soares_senescent_fibroblasts_NbHSF; Human umbilical vein endothelial cells, IL-4 induced; Soares_parathyroid_tumor_NbHPA; Spinal cord; Human fetal heart, Lambda ZAP Express; NCI_CGAP_Kid5; Soares_parathyroid_tumor_NbHPA; Hemangiopericytoma; Soares breast 2NbHBst;

- Human Adrenal Gland Tumor; NCI_CGAP_Kid3; NCI_CGAP_Pan1;
 NCI_CGAP_Brn23; Soares_testis_NHT; Soares_fetal_heart_NbHH19W;
 Soares_placenta_8to9weeks_2NbHP8to9W; Stratagene NT2 neuronal precursor
 937230; Hepatocellular Tumor, re-excision; Human Liver, normal; Human T-Cell
 5 Lymphoma; Soares breast 3NbHBst; Human Testes Tumor; Human fetal heart,
 Lambda ZAP Express; Normal colon; Soares melanocyte 2NbHM; Primary Dendritic
 cells, frac 2; Human Fetal Lung III; Human Amygdala; Normal Human Trabecular
 Bone Cells; Soares_NhHMPu_S1; Soares_NFL_T_GBC_S1;
 Soares_total_fetus_Nb2HF8_9w; Soares_fetal_liver_spleen_1NFLS_S1 and Human
 10 Cerebellum.

- Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are
 related to SEQ ID NO:33 and may have been publicly available prior to conception of
 the present invention. Preferably, such related polynucleotides are specifically
 15 excluded from the scope of the present invention. To list every related sequence
 would be cumbersome. Accordingly, preferably excluded from the present invention
 are one or more polynucleotides comprising a nucleotide sequence described by the
 general formula of a-b, where a is any integer between 1 to 1949 of SEQ ID NO:33, b
 is an integer of 15 to 1963, where both a and b correspond to the positions of
 20 nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a
 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 24

- The computer algorithm BLASTX has been used to determine that the
 25 translation product of this gene shares sequence homology with, as a non-limiting
 example, the sequence accessible through the following database accession no.

gi|3643809 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "zinc finger protein 216 splice variant 1 [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >gi|3643809 (AF062346) zinc finger protein 216 splice variant 1 [Homo
      sapiens] >gi|3643811 (AF062347) zinc finger protein 216 splice variant 2
      [Homo sapiens] >gi|3668066 (AF062072) zinc finger protein 216 [Homo
10     sapiens] >sp|076080|076080 ZINC FINGER PROTEIN 216. >bbs|165969
      ubiquitin homolog [cattle, endothelial cells, Peptide Partial, 46 aa] [Bos
      taurus] (SUB 168-213)
      Length = 213

      Plus Strand HSPs:

15     Score = 577 (203.1 bits), Expect = 2.7e-55, P = 2.7e-55
      Identities = 120/213 (56%), Positives = 142/213 (66%), Frame = +3

20     Q:  363 MAQETNHSQVPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQNSNGRISPPAT---SVSS 533
      MAQETN + PMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQ +S GR+SP T S S
      S:  1 MAQETNQTGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQNS-GRMSPMGTASGSNSP 59

      Q:  534 LSES L P V Q C T D G S V P E A Q S A L D X X X X X X X X X X L L S E --SVASSQLDSTSVDKAVP 707
      S+S VQ D S+ + A + + ++ S+ D + K
25     S:  60 TSDSASVQRADTSLNNCCEGAAGTSEKSRNVPVAALPVTQQMTESISREDKITTPKT-E 118

      Q:  708 ETEDVQASVSDTAQQPSEEQS---KSLEKPKQKKNRCFCMRKKVGLTGFECRCGNVYCGVH 881
      +E V S + QPS QS K+ E PK KKNRCFCMRKKVGLTGF+CRCGN++CG+H
30     S:  119 VSEPVTQPSVSPSQSTSQSEKKAPELPKPKKNRCFCMRKKVGLTGFDCRCGNLFCGLH 178

      Q:  882 RYSDVHNCSYNYKADAAEKIRKENPVVVGEKIQKI 986
      RYSD HNC Y+YKA+AA KIRKENPVVV EKIQ+I
      S:  179 RYSDKHNCPPYDYKAEAAAKIRKENPVVVAEKIQRI 213

```

35 The segment of gi|3643809 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 143. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have

40 been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 144 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- 5 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Testes and to a lesser extent in Soares retina N2b4HR; Human Osteoclastoma; Human Testes, Reexcision; Morton Fetal Cochlea; NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Soares_pregnant_uterus_NbHPU; Soares infant brain 1NIB;
- 10 Soares_pregnant_uterus_NbHPU; Stratagene ovarian cancer (#937219); Stratagene neuroepithelium NT2RAMI 937234; Human Activated Monocytes; Messangial cell, frac 2; Human Adult Small Intestine; Human Umbilical Vein, Reexcision; Soares_testis_NHT; Ovarian Tumor 10-3-95; Soares_NhHMPu_S1; Pancreas Islet Cell Tumor; 12 Week Old Early Stage Human; Adipocytes; Soares melanocyte
- 15 2NbHM; Human Adult Pulmonary, re-excision; Endothelial cells-control; Soares_testis_NHT; Human Bone Marrow, treated; Osteoblasts; Keratinocyte; Soares_fetal_lung_NbHL19W; A-14 cell line; Human Colon Carcinoma (HCC) cell line; Human Thyroid; Human T-cell lymphoma, re-excision; Stratagene colon HT29 (#937221); Stratagene ovary (#937217); Human Lung Cancer, re-excision; Morton
- 20 Fetal; H. Kidney Cortex, subtracted; Human Osteoclastoma, re-excision; Soares_fetal_lung_NbHL19W; Soares_parathyroid_tumor_NbHPA; H. Ovarian Tumor, II, OV5232; Jurkat T-Cell, S phase; Testis I; normalized infant brain cDNA; L428; human ovarian cancer; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Human Umbilical Vein Endothelial Cells, uninduced; Human
- 25 Pancreas Tumor; Human umbilical vein endothelial cells, IL-4 induced; Human Placenta (re-excision); Atrium cDNA library Human heart; Human Activated T-Cells,

re-excision; Synovial Fibroblasts (control); Human Thymus Stromal Cells; Human Adrenal Gland Tumor; CHME Cell Line, treated 5 hrs; Hepatocellular Tumor, re-excision; Human Gall Bladder; Smooth muscle, serum treated; Human Testes Tumor; Human Fetal Lung III; 12 Week Early Stage Human II, Reexcision;

- 5 Soares_pineal_gland_N3HPG; Bone marrow; Smooth muscle, control; HUMAN B CELL LYMPHOMA; Spleen, Chronic lymphocytic leukemia; Hodgkin's Lymphoma II; Activated T-cell(12h)/Thiouridine-re-excision; Human fetal heart, Lambda ZAP Express; Human 8 Week Whole Embryo; Human Cerebellum and Primary Dendritic Cells, lib 1.

- 10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:34 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
- 15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1753 of SEQ ID NO:34, b is an integer of 15 to 1767, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a
- 20 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 25

- The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting
- 25 example, the sequence accessible through the following database accession no. gil1519392 (all information available through the recited accession number is

incorporated herein by reference) which is described therein as "leptin receptor [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >gi|1519392 leptin receptor [Homo sapiens] >sp|Q92920|Q92920 LEPTIN
      RECEPTOR.
          Length = 958

      Plus Strand HSPs:
10      Score = 339 (119.3 bits), Expect = 6.6e-27, Sum P(2) = 6.6e-27
      Identities = 63/69 (91%), Positives = 65/69 (94%), Frame = +1

      Q: 1543 KQMLEGSMVLKSHHHSLISSTQGHKHCGRPQGGLPRKTRDLCSLVYLLTFPPLLSYDPAK 1722
          ++MLEGSM VKSHHHSLISSTQGHKHCGRPQGGL RKTRDLCSLVYLLT PPLLSYDPAK
15      S: 890 KQMLEGSMFVKSHHHSLISSTQGHKHCGRPQGGLHRKTRDLCSLVYLLTFPPLLSYDPAK 949

      Q: 1723 SPSARNTQE 1749
          SPS RNTQE
20      S: 950 SPSVRNTQE 958

      Score = 44 (15.5 bits), Expect = 6.6e-27, Sum P(2) = 6.6e-27
      Identities = 7/14 (50%), Positives = 10/14 (71%), Frame = +3

25      Q: 537 DWSAHLIFTMQDLL 578
          DWS +FT QD++
      S: 321 DWSTPRVFTTQDVI 334
  
```

- The segments of gi1519392 that are shown as "S" above are set out in
- 30 the sequence listing as SEQ ID NO. 145 and SEQ ID NO. 147. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.
- 35 Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 146 and/or SEQ ID NO. 148 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: L1 Cell line; Spleen metastatic melanoma; Neutrophils IL-1 and LPS induced and to a lesser extent in Human Testes; A-14 cell line; Human Prostate Cancer, Stage C fraction; H. Kidney Cortex, subtracted; Human Primary
- 5 Breast Cancer Reexcision; CD34 depleted Buffy Coat (Cord Blood), re-excision; Spleen, Chronic lymphocytic leukemia; human colon cancer; Human Pineal Gland; CD34 positive cells (cord blood),re-ex; Human Stomach,re-excision; Jurkat T-cell G1 phase; Human Fetal Dura Mater; Human Ovary; Human Adipose; breast lymph node CDNA library; Early Stage Human Brain; Human Fetal Lung III; Human
 - 10 Microvascular Endothelial Cells, fract. A; CD34 positive cells (Cord Blood); H. Frontal cortex,epileptic,re-excision; neutrophils control; Primary Dendritic Cells, lib 1; Human Fetal Brain; Prostate,BPH, Lib 2; Larynx carcinoma II; Larynx carcinoma III; A1-CELL LINE; Human Fetal Brain; stomach cancer (human); SKIN; Brain-medulloblastoma; H. Epididymus, caput & corpus; H. Epididymus, cauda; Healing
 - 15 groin wound - zero hr post-incision (control); Stomach cancer (human),re-excision; Human Hypothalamus,schizophrenia, re-excision; Human Colon Cancer,re-excision; Alzheimers, spongy change; Healing groin wound, 7.5 hours post incision; Human Osteosarcoma; Synovial hypoxia; Synovial Fibroblasts (II1/TNF), sub; H. Meningioma, M1; KMH2; Human Thymus; Human Activated T-Cells; Human Heart;
 - 20 Merkel Cells; Liver, Hepatoma; Human Chondrosarcoma; Human Thymus; Human Liver, normal; Human Gall Bladder; PC3 Prostate cell line; Neutrophils IL-I and LPS induced; Dendritic cells, pooled; Primary Dendritic cells,frac 2; Human Testes, Reexcision; human tonsils; Human Fetal Heart; Anergic T-cell; Human Amygdala; Human Bone Marrow, treated; Bone Marrow Cell Line (RS4,11); Hodgkin's
 - 25 Lymphoma II; Nine Week Old Early Stage Human and Human Cerebellum.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1803 of SEQ ID NO:35, b is an integer of 15 to 1817, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 26

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi4153872 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "structure confirmed by Genscan, human EST AA447021 (NID:g2159686) and mouse EST AA119040 (NID:g1676735) [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

>gi4153872 (AC004918) structure confirmed by Genscan, human EST AA447021
(NID:g2159686) and mouse EST AA119040 (NID:g1676735) [Homo sapiens]
Length = 180

Plus Strand HSPs:

Score = 243 (85.5 bits), Expect = 7.9e-19, P = 7.9e-19
Identities = 46/47 (97%), Positives = 47/47 (100%), Frame = +1

```

Q: 973 PQGAGGSFSDSEYEAMPVEVKLLPRKLQFFCDPRKREQMLTSPTQ 1113
 P+GAGGSFSDSEYEAMPVEVKLLPRKLQFFCDPRKREQMLTSPTQ
 S: 134 PEGAGGSFSDSEYEAMPVEVKLLPRKLQFFCDPRKREQMLTSPTQ 180

5 The segment of gil4153872 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 149. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have
 10 been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 150 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

15 When tested against Jurkat cell lines, supernatants removed from cells containing this gene activated the NF- κ B transcription factor. Thus, it is likely that this gene activates Jurkat cells by activating a transcriptional factor found within these cells. Nuclear factor κ B is a transcription factor activated by a wide variety of agents, leading to cell activation, differentiation, or apoptosis. Reporter constructs utilizing
 20 the NF- κ B promoter element are used to screen supernatants for such activity.

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain INIB and to a lesser extent in NCI_CGAP_AA1; Stratagene ovarian cancer (#937219); Synovial hypoxia-RSF subtracted; Soares_fetal_heart_NbHH19W; Soares_multiple_sclerosis_2NbHMSP;
 25 Human Placenta; Gessler Wilms tumor; Soares_fetal_liver_spleen_INFLS_S1; Soares_NhHMPu_S1; Human Pancreatic Langerhans; CD34+ cell, I, frac II; NCI_CGAP_HN3; NCI_CGAP_Co12; NCI_CGAP_Kid5; Stratagene hNT neuron

- (#937233); Saos2, Dexamethosome Treated; Human T-cell lymphoma, re-excision; Stratagene neuroepithelium (#937231); Smooth Muscle- HASTE normalized; NTERA2 + retinoic acid, 14 days; Healing groin wound, 7.5 hours post incision; KMH2; Human Umbilical Vein Endothelial Cells, uninduced; Human Hippocampus;
- 5 Human Chondrosarcoma; H Macrophage (GM-CSF treated), re-excision; Soares_total_fetus_Nb2HF8_9w; Soares_parathyroid_tumor_NbHPA; Stratagene lung carcinoma 937218 and Colon Normal III.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
- 10 related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
- 15 general formula of a-b, where a is any integer between 1 to 2474 of SEQ ID NO:36, b is an integer of 15 to 2488, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.

20 FEATURES OF PROTEIN ENCODED BY GENE NO: 27

- The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi|307307 (all information available through the recited accession number is
- 25 incorporated herein by reference) which is described therein as "neuroendocrine-

specific protein A [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

5   >gi|307307 nueroendocrine-specific protein A [Homo sapiens]
   >pir|A46583|A46583
      neuroendocrine-specific protein A - human >sp|Q16799|Q16799
      NEUROENDOCRINE-SPECIFIC PROTEIN A (NEUROENDOCRINE-SPECIFIC
PROTEIN
10  {SUB
      B). >gi|307309 nueroendocrine-specific protein B [Homo sapiens]
      421-776}
      Length = 776

      Plus Strand HSPs:

15  Score = 446 (157.0 bits), Expect = 3.2e-43, Sum P(2) = 3.2e-43
      Identities = 91/202 (45%), Positives = 115/202 (56%), Frame = +2

20  Q: 146 PAASEPVI--RSSAVVDLLYWRDIKTKGXXXXXXXXXXXXXXXXXXXXXTAYIALALLSV 319
      P A P++ +DLLYWRDIK+TG AY+ALA LS
      S: 575 PGAPPPLFLNKKAKIDLLYWRDIKQTGIVFGSFLLLFSLTQFSVSVSVVAYLALAALS 634

      Q: 320 TISPRIYKGVIAIQKIDEGHPPFAYLSEVAISEELVQKYSNSALGHVNMCTIKEXXXXX 499
      TISPRIYK V+QA+QK+DEGHPP+AYLE E+ +S+E +QKY++ +VN T+KE
25  S: 635 TISPRIYKSVLQAVQKTDGHPFPKAYLELITLSQEIQKTYTDCLQFYVNSTLKLRLRF 694

      Q: 500 XXXXXXXXXXKFAVLMWVFTYVXXXXXXXXXXXXXXXXXXXXXVPVIYERHQAQIDHYLGLAN 679
      KFAVLMW+ TTV +PV+Y +HQAQID YLGL
      S: 695 LVQDLVDSLKFAVLMWLLTYVGALFNGLTLLMAVVSMTLFPVVIVKHQAQIDQYLGIVR 754

30  Q: 680 KNVKDAMAKIQAKIPGLKRRKAE 745
      ++ +AKIQAKIPG KR AE
      S: 755 THINAVVAKIQAKIPGAKRHA 776

```

35 The segment of gi|307307 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 151. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been

40 described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 152 which

corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Osteoblasts and to a lesser extent in Soares placenta Nb2HP;

- 5 Soares_senescent_fibroblasts_NbHSF; Primary Dendritic Cells, lib 1; Human Thymus Stromal Cells; Morton Fetal Cochlea; Smooth muscle, serum treated; Keratinocyte; Stratagene fetal spleen (#937205); Bone Marrow Stromal Cell, untreated; Smooth muscle, serum induced, re-exc; Soares melanocyte 2NbHM; Nine Week Old Early Stage Human; Synovial Fibroblasts (control); Stratagene lung
- 10 (#937210); NCI_CGAP_Kid5; Stromal cell TF274; Human Eosinophils; Human Amygdala; Soares fetal liver spleen 1NFLS; Soares infant brain 1N1B; Stratagene pancreas (#937208); Human Umbilical Vein Endothelial Cells, uninduced; Soares_senescent_fibroblasts_NbHSF; Epithelial-TNF α and INF induced; H Macrophage (GM-CSF treated), re-excision; Colon Tumor II; Normal colon;
- 15 Soares_fetal_heart_NbHH19W; Human Microvascular Endothelial Cells, fract. A; NCI_CGAP_Lu5; Hodgkin's Lymphoma II; Soares_pregnant_uterus_NbHPU; Soares_fetal_lung_NbHL19W; Smooth muscle, control, re-excision; Synovial IL-1/TNF stimulated; Human Amygdala, re-excision; Stratagene muscle 937209; Synovial hypoxia; Healing groin wound, 6.5 hours post incision; Temporal cortex-
- 20 Alzheimer, subtracted; Macrophage-oxLDL; Soares_parathyroid_tumor_NbHPPA; Human umbilical vein endothelial cells, IL-4 induced; Human Adrenal Gland Tumor; CHME Cell Line, treated 5 hrs; Macrophage-oxLDL, re-excision; CHME Cell Line, untreated; Adipocytes; Human Osteoclastoma; Human 8 Week Whole Embryo; Soares_parathyroid_tumor_NbHPPA; NCI_CGAP_GCB1; H. Adipose Tissue; Smooth
- 25 Muscle Serum Treated, Norm; Soares retina N2b4HR; Human endometrial stromal cells-treated with estradiol; Amniotic Cells - Primary Culture; Healing groin wound,

- 7.5 hours post incision; Human Osteoclastoma, re-excision;
 Soares_pregnant_uterus_NbHPU; Synovial Fibroblasts (III/TNF), subt;
 STRATAGENE Human skeletal muscle cDNA library, cat. #936215.; Human
 Umbilical Vein, Reexcision; Soares_total_fetus_Nb2HF8_9w; human ovarian cancer;
- 5 Human Osteoblasts II; Soares_fetal_heart_NbHH19W; Human Testes Tumor, re-
 excision; Soares_pineal_gland_N3HPG; Pancreas Islet Cell Tumor; 12 Week Old
 Early Stage Human; Human Substantia Nigra; Soares_pregnant_uterus_NbHPU;
 Dendritic cells, pooled; Human Fetal Kidney, Reexcision; CD34 depleted Buffy Coat
 (Cord Blood), re-excision; Colon Normal III; Smooth muscle, control; Stratagene
- 10 pancreas (#937208); T Cell helper I; H. Frontal cortex, epileptic, re-excision;
 Stratagene neuroepithelium NT2RAMI 937234; Soares_fetal_heart_NbHH19W;
 Soares_pregnant_uterus_NbHPU; Human Placenta, subtracted; NCI_CGAP_Pr1;
 Soares_multiple_sclerosis_2NbHMSP; Human Aortic Endothelium; Human
 Cerebellum, subtracted; Adipocytes, re-excision; H. Epididymus, cauda; Human
- 15 Pineal Gland; Morton Fetal; Human Synovium; Stratagene hNT neuron (#937233);
 Hepatocellular Tumor; Human Prostate Cancer, Stage C fraction; H Female Bladder,
 Adult; NTERA2 + retinoic acid, 14 days; H. Kidney Cortex, subtracted;
 Glioblastoma; Human Whole Brain #2 - Oligo dT > 1.5Kb; Stratagene endothelial
 cell 937223; Prostate BPH; Spinal Cord, re-excision; Human Prostate;
- 20 NCI_CGAP_GCB1; L428; 12 Week Old Early Stage Human, II; HUMAN JURKAT
 MEMBRANE BOUND POLYSOMES; NCI_CGAP_Br2; Human
 Hypothalamus, Schizophrenia; Soares_testis_NHT; Human Pancreas Tumor,
 Reexcision; Human Hippocampus; Human Adipose; NCI_CGAP_GCB1; Rejected
 Kidney, lib 4; Soares_parathyroid_tumor_NbHPA; Stratagene liver (#937224); Colon
- 25 Tumor; Soares_fetal_lung_NbHL19W; Colon Normal II; Soares breast 3NbHBst;
 Early Stage Human Brain; normalized infant brain cDNA; Primary Dendritic

- cells, frac 2; 12 Week Early Stage Human II, Reexcision; Human Placenta;
 Endothelial cells-control; Stratagene colon HT29 (#937221); Stratagene ovarian
 cancer (#937219); NCI_CGAP_Co8; NCI_CGAP_Brn23; Human fetal heart, Lambda
 ZAP Express; NCI_CGAP_GC4; NCI_CGAP_Lu5; NCI_CGAP_Alvl1;
- 5 NCI_CGAP_Kid3; NCI_CGAP_Kid5; NCI_CGAP_Pr22; NCI_CGAP_Brn23;
 Stratagene muscle 937209; Stratagene colon (#937204); Human Cerebellum; Human
 Old Ovary, subtracted; Human Fetal Kidney; Human Prostate, differential expression;
 Uterus, normal; Human Astrocyte; Human Infant Adrenal Gland, subtracted; Human
 Old Ovary; Early Stage Human Brain, random primed; Soares ovary tumor NbHOT;
- 10 Colon Normal; Human Amygdala Depression, re-excision; Human rejected kidney;
 NCI_CGAP_Alvl1; NCI_CGAP_GCB1; Larynx Tumor; Human Umbilical Vein
 Endothelial cells, frac B, re-excision; H. hypothalamus, frac A; H. Striatum
 Depression, subtracted; Ku 812F Basophils Line; Human 8 Week Whole Embryo,
 subtracted; Hypothalamus; Soares_fetal_lung_NbHL19W; Soares retina N2b5HR;
- 15 Human OB HOS treated (10 nM E2) fraction I; Human colon carcinoma (HCC) cell
 line, remake; H. Atrophic Endometrium; HSC172 cells; Healing Abdomen
 wound, 70&90 min post incision; Frontal lobe, dementia, re-excision; Supt Cells,
 cyclohexamide treated; H. Epididymus, caput & corpus; Aorta endothelial cells +
 TNF-a; Invasive poorly differentiated lung adenocarcinoma, metastatic; Amniotic
- 20 Cells - TNF induced; Human Lung; HSA 172 Cells; Stratagene pancreas (#937208);
 NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Human Normal Breast;
 Messangial cell, frac 2; H. Whole Brain #2, re-excision; Stomach cancer (human), re-
 excision; Human Colon Cancer, re-excision; Soares_pregnant_uterus_NbHPU; pBMC
 stimulated w/ poly I/C; Smooth muscle, IL1b induced;
- 25 Soares_fetal_lung_NbHL19W; Human Adipose Tissue, re-excision; Human
 Osteosarcoma; Human endometrial stromal cells; Stratagene fetal retina 937202;

- Stratagene ovarian cancer (#937219); Stratagene neuroepithelium (#937231);
 Stratagene colon (#937204); Stratagene neuroepithelium NT2RAMI 937234;
 Myeloid Progenitor Cell Line; Spleen metastatic melanoma; Stratagene muscle 937209;
 Brain Frontal Cortex, re-excision; Human Infant Brain; Human Chronic Synovitis; H.
- 5 Kidney Medulla, re-excision; Human promyelocyte; normalized infant brain cDNA;
 Human Bone Marrow, re-excision; KMH2; Human Brain, Striatum;
 NCI_CGAP_Alv1; NCI_CGAP_Kid5; NCI_CGAP_Pr22; Monocyte activated, re-
 excision; Human Fetal Kidney; NCI_CGAP_GC2; NCI_CGAP_Co11; Human
 Activated T-Cells; T-Cell PHA 24 hrs; Barstead spleen HPLRB2; Gessler Wilms
- 10 tumor; Human pancreatic islet; Soares_pregnant_uterus_NbHPU;
 Soares_total_fetus_Nb2HF8_9w; Olfactory epithelium,nasalcavity;
 Soares_multiple_sclerosis_2NbHMSP; Spinal cord; Human Activated T-Cells, re-
 excision; Human Chondrosarcoma; PERM TF274; Soares_fetal_lung_NbHL19W;
 Stratagene neuroepithelium (#937231); NCI_CGAP_Ew1; NCI_CGAP_Pr10; Soares
- 15 breast 2NbHBst; Fetal Liver, subtraction II; NTERA2, control; STRATAGENE
 Human skeletal muscle cDNA library, cat. #936215.; Soares_testis_NHT;
 Soares_fetal_heart_NbHH19W; Soares_total_fetus_Nb2HF8_9w; Stratagene fetal
 retina 937202; Stratagene hNT neuron (#937233); Human Liver, normal; Human Gall
 Bladder; NCI_CGAP_GC4; PC3 Prostate cell line; Resting T-Cell Library,II; Human
- 20 T-Cell Lymphoma; Human pancreatic islet; Jia bone marrow stroma;
 Soares_testis_NHT; Soares_total_fetus_Nb2HF8_9w; Stratagene lung carcinoma
 937218; Colon Carcinoma; Human Placenta; NCI_CGAP_Pr25;
 Soares_multiple_sclerosis_2NbHMSP; Stratagene cat#937212 (1992); Stratagene
 fetal retina 937202; Stratagene hNT neuron (#937233); Neutrophils control, re-
 25 excision; Human Testes, Reexcision; Endothelial-induced; Human Adult
 Pulmonary,re-excision; NCI_CGAP_Lu5; NCI_CGAP_Kid5; NCI_CGAP_Kid6;

Monocyte activated; Spleen, Chronic lymphocytic leukemia; Stratagene neuroepithelium (#937231); Neutrophils IL-1 and LPS induced; NCI_CGAP_Ut1; NCI_CGAP_Ov36; NCI_CGAP_Brn35; NCI_CGAP_Kid12; Activated T-cell(12h)/Thiouridine-re-excision; neutrophils control; Clontech human aorta polyA+ mRNA (#6572); HM1; NCI_CGAP_AA1; NCI_CGAP_Br3; NCI_CGAP_Ew1; NCI_CGAP_HN4; NCI_CGAP_Kid6; NCI_CGAP_Lar1; NCI_CGAP_PNS1; NCI_CGAP_Pr12; NCI_CGAP_Pr24; NCI_CGAP_Brn25; Stratagene pancreas (#937208) and NCI_CGAP_GCB1.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1744 of SEQ ID NO:37, b is an integer of 15 to 1758, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 28

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil3002527 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "neuronal thread

protein AD7c-NTP [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >gi|3002527 (AF010144) neuronal thread protein AD7c-NTP [Homo sapiens]
      >sp|O60448|O60448 NEURONAL THREAD PROTEIN AD7C-NTP.
      Length = 375

      Minus Strand HSPs:
10     Score = 189 (66.5 bits), Expect = 1.6e-12, Sum P(2) = 1.6e-12
      Identities = 39/56 (69%), Positives = 42/56 (75%), Frame = -1

      Q:   813 HSVTQAAMPWRDLGSLQAPPPGFMPPFSCLSLPGSWDYRCPPPPANFFVFLVETGF 646
      +SVTQA + WR+LGSLQ PPGF FSC SL SWDYR PP ANFFVFLVE GF
15     S:   207 NSVTQAGVQWRNLGSLQLPPLPGFKLFSCPSLLSSWDYR-RFPRLANFFVFLVEMGF 261

```

The segment of gi|3002527 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 153. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities.

20 Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 154 which
 25 corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: PERM TF274 and to a lesser extent in Human Umbilical Vein Endothelial Cells, uninduced.

30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

- excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1144 of SEQ ID NO:38, b is an integer of 15 to 1158, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 29

- The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi403460 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "transformation-related protein [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

>gi403460 transformation-related protein [Homo sapiens] >sp|Q15662|Q15662
TRANSFORMATION-RELATED PROTEIN (FRAGMENT).
Length = 368

Minus Strand HSPs:

Score = 160 (56.3 bits), Expect = 5.3e-08, P = 5.3e-08
Identities = 42/92 (45%), Positives = 49/92 (53%), Frame = -3

Q: 2140 VLLPRLECSGSSLAH*NLCPP----VQAIPLPQTPEQPGPQAPITTPGQP-FVFLVQTGF 1976
      +L PRLEC+G LAH L P + LP + + P P Q FVF V+TGF
S: 5 LLSPLRECNMILAHCKLRLPGFKRFSCLSLPSSWDYR--HVP---PRQVHFVFSVETGF 59

Q: 1975 HHADQAGPEXXXXXX-XXXXPKCWDYRREHHAMPAL 1868
      H A QAG E PKCWDYRR+ ANP L
S: 60 HRAGQAGLELLTSSVPPTSAFFKPCWDYRRDDQAMPTL 96

```


The segment of gil403460 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 155. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 156 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Prostate BPH, re-excision; Whole 6 Week Old Embryo; HL-60, PMA 4H, re-excision; L428; human tonsils; Barstead spleen HPLRB2; HUMAN B CELL LYMPHOMA.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 88 as residues: Cys-64 to Lys-70. Polynucleotides encoding said polypeptides are also provided.

When tested against sensory neuron cell lines, supernatants removed from cells containing this gene activated the EGR1 assay. Thus, it is likely that this gene activates sensory neuron cells through a signal transduction pathway. Early growth response 1 (EGR1) is a promoter associated with certain genes that induces various tissues and cell types upon activation, leading the cells to undergo differentiation and proliferation.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:39 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

5 general formula of a-b, where a is any integer between 1 to 2176 of SEQ ID NO:39, b is an integer of 15 to 2190, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.

10 FEATURES OF PROTEIN ENCODED BY GENE NO: 30

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi|3094014 (all information available through the recited accession number is

15 incorporated herein by reference) which is described therein as "Homo sapiens protein". A partial alignment demonstrating the observed homology is shown immediately below.

```

20 >gi|3094014 (AF060862) unknown [Homo sapiens]
    Length = 119

    Plus Strand HSPs:

    Score = 266 (93.6 bits), Expect = 2.6e-21, P = 2.6e-21
25 Identities = 50/101 (49%), Positives = 67/101 (66%), Frame = +3

    Q:  534  RLFSCANCDTILTNRSELSTRFTGATGRAFLFNKVVNLQYSEVQDRVMLTGRHMVRDVS 713
        R +SC +C  L N  ELIS  F G+ GRA+LFN VVN+  ++RV+LTG H V D+
    S:  19  RTYSCHCRAHLANHDELISKSFQGSQGRAYLFNSVVNVGCGPAEERVLTLGLHAVADIY 78

    Q:  714  CKNCNCKLGIWIEFATEDSQRYKEGRVILERALVRESEGE 836
        C+NC + LGW YE A E SQ+YKEG+ I+E A + +  G+E
30 S:  79  CENCKTTLGWKYEHAPESSQKYKEGKFIIELAHMIKDNQWE 119
  
```

The segment of gil3094014 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 157. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein.

- 5 Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

- Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 158 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares_parathyroid_tumor_NbHPA and to a lesser extent in Human Testes; Primary Dendritic Cells, lib 1; Soares infant brain 1NIB; Human Neutrophil; Soares_pregnant_uterus_NbHPU; Soares fetal liver spleen 1NFLS;
- 15 Soares adult brain N2b5HB55Y; Soares placenta Nb2HP; Soares_pregnant_uterus_NbHPU; Resting T-Cell Library,II; Soares melanocyte 2NbHM; Primary Dendritic cells,frac 2; Human Bone Marrow, treated; Neutrophils IL-1 and LPS induced; Morton Fetal Cochlea; Soares_placenta_8to9weeks_2NbHP8to9W; Soares_pregnant_uterus_NbHPU;
- 20 Human Osteoclastoma, re-excision; Stromal cell TF274; Human Adrenal Gland Tumor; Human Gall Bladder; Human T-Cell Lymphoma; Soares_fetal_lung_NbHL19W; Smooth muscle, serum treated; Dendritic cells, pooled; Human Placenta; Human Amygdala; Soares_pregnant_uterus_NbHPU; Monocyte activated; H. Frontal cortex,epileptic,re-excision;
- 25 Soares_fetal_liver_spleen_INFLS_S1; NCI_CGAP_GCB1; H Amygdala Depression, subtracted; Kidney medulla; Healing Abdomen Wound,15 days post incision;

- Colorectal Tumor; Smooth muscle-ILb induced; Activated T-cells; Soares retina N2b4HR; Human Pineal Gland; Human adult small intestine, re-excision; Apoptotic T-cell, re-excision; Morton Fetal; Human Synovium; Human Prostate Cancer, Stage C fraction; NTERA2 + retinoic acid, 14 days; Glioblastoma; Healing groin wound,
- 5 6.5 hours post incision; Soares_placenta_8to9weeks_2NbHP8to9W; T-Cell PHA 16 hrs; Soares_senescent_fibroblasts_NbHSF; Human Bone Marrow, re-excision; Human Brain, Striatum; Human Heart; Stratagene neuroepithelium NT2RAM1 937234; Human Adipose; Bone Marrow Stromal Cell, untreated; Rejected Kidney, lib 4; Stratagene endothelial cell 937223; Stratagene ovarian cancer (#937219);
- 10 Macrophage (GM-CSF treated); Human Liver, normal; Soares_fetal_heart_NbHH19W; Pancreas Islet Cell Tumor; 12 Week Old Early Stage Human; Neutrophils IL-1 and LPS induced; Human Eosinophils; Soares breast 3NbHBst; Early Stage Human Brain; Soares_placenta_8to9weeks_2NbHP8to9W; Human Testes, Reexcision; human tonsils; Human Neutrophil, Activated;
- 15 Endothelial-induced; Anergic T-cell; Human Microvascular Endothelial Cells, fract. A; Soares_fetal_lung_NbHL19W; Soares_senescent_fibroblasts_NbHSF; neutrophils control; Osteoblasts; Keratinocyte; Human 8 Week Whole Embryo; Nine Week Old Early Stage Human; Human Cerebellum and Stratagene hNT neuron (#937233).

- Preferred polypeptides of the present invention comprise immunogenic
- 20 epitopes shown in SEQ ID NO: 89 as residues: Asn-2 to Trp-13. Polynucleotides encoding said polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of
- 25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2562 of SEQ ID NO:40, b is an integer of 15 to 2576, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 31

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gnll|PID|e1288408 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "(AJ005678) SA3 [Mus musculus]". A partial alignment demonstrating the observed homology is shown immediately below.

```

>gnl|PID|e1288408 (AJ005678) SA3 [Mus musculus] >sp|O70576|O70576 NUCLEAR
    PROTEIN SA3.
    Length = 1240

    Plus Strand HSPs:

    Score = 121 (42.6 bits), Expect = 4.2e-10, Sum P(3) = 4.2e-10
    Identities = 22/37 (59%), Positives = 27/37 (72%), Frame = +2

    Q:  527  RNMEGVLMVDVDCESVYPIV*ASN*GLASAVGEFLYWK 637
        +NMEGVLM DCE +Y IV SN +AS+ GEF+YWK
    S:  430  KNMEGVLTADCEKIYSIVYISNRMASSAGEFVYWK 466

    Score = 92 (32.4 bits), Expect = 4.2e-10, Sum P(3) = 4.2e-10
    Identities = 19/49 (38%), Positives = 27/49 (55%), Frame = +2

    Q:  899  RLFYFECEIRTMGGREQRQSPGAQRTFFQLLLLC-GEQANRXLLQAD 1042
        ++F+PEC  + + RE+R+SP AQ+TF LLL E N D
    S:  466  KIFHPECGAKAVSDRERRRSPQAQKTFIYLLAFMESEHHNHAAYLVD 514
  
```

The segments of gnl|PIdle|288408 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 159 and SEQ ID NO. 161. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 160 and/or SEQ ID NO. 162 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Cerebellum and to a lesser extent in Fetal Heart; Human Synovial Sarcoma; Chromosome 7 Fetal Brain cDNA Library; Bone Marrow Cell Line (RS4,11); Human Activated T-Cells; Human Adult Testes, Large Inserts, Reexcision; Macrophage-oxLDL; breast lymph node CDNA library; Colon Normal II; Endothelial-induced; Activated T-Cell (12hs)/Thiouridine labelledEco; CD34 depleted Buffy Coat (Cord Blood), re-excision; HUMAN B CELL LYMPHOMA; Soares fetal liver spleen INFLS; Human Fetal Brain,normalized c50F; Soares_NFL_T_GBC_S1; Soares ovary tumor NbHOT; CD34+ cell, I; HL-60, RA 4h, Subtracted; H. Epididymus, caput & corpus; H. cerebellum, Enzyme subtracted; H. Whole Brain #2, re-excision; LNCAP prostate cell line; Myeloid Progenitor Cell Line; Healing groin wound, 6.5 hours post incision; wilm's tumor; H. Lymph node breast Cancer; CD34 depleted Buffy Coat (Cord Blood); Human Dermal Endothelial Cells,untreated; Monocyte activated, re-excision; Apoptotic T-cell; Human Pancreas Tumor; T-Cell PHA 24 hrs; Human Heart; Spinal cord; Human Rhabdomyosarcoma; Human Thymus; Hemangiopericytoma; Soares_testis_NHT; Ovarian Tumor 10-3-95;

NCI_CGAP_GCB1; Smooth muscle, serum induced, re-exc; Macrophage-oxLDL, re-excision; NCI_CGAP_Co10; NCI_CGAP_Kid3; NCI_CGAP_Kid5; Stratagene pancreas (#937208); Resting T-Cell Library, II; Human Eosinophils; 12 Week Early Stage Human II, Reexcision; Human Fetal Heart; Endothelial cells-control; Colon
5 Normal III; Anergic T-cell; Human Bone Marrow, treated; T Cell helper I; Activated T-cell(12h)/Thiouridine-re-excision and T cell helper II.

When tested against sensory neuron cell lines, supernatants removed from cells containing this gene activated the EGR1 assay. Thus, it is likely that this gene activates sensory neuron cells through a signal transduction pathway. Early growth
10 response 1 (EGR1) is a promoter associated with certain genes that induces various tissues and cell types upon activation, leading the cells to undergo differentiation and proliferation.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
15 related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
20 general formula of a-b, where a is any integer between 1 to 1138 of SEQ ID NO:41, b is an integer of 15 to 1152, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 32

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

- 5 gi|2088839 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "(AF003386) No definition line found [Caenorhabditis elegans]". A partial alignment demonstrating the observed homology is shown immediately below.

```

10  >gi|2088839 (AF003386) No definition line found [Caenorhabditis elegans]
    >sp|O01894|O01894 CODED FOR BY C. ELEGANS CDNA YK53D12.5.
    Length = 527

    Minus Strand HSPs:

15  Score = 640 (225.3 bits), Expect = 3.1e-101, Sum P(4) = 3.1e-101
    Identities = 115/220 (52%), Positives = 156/220 (70%), Frame = -2

20  Q: 3657 PWPXNLCTKCQPSAITLNRQKYRHVDNIMFENHTVADRFLDFWRKTNQHGFGYLYGRYTE 3478
    P+P +CTKC+P +TLNRQK+RHDVNI EN ++FLD+WR +G+Q G+L G+Y
    S: 102 PFPKGICTCKPQVVTLNRQKFRHVDNIQIENQELVNQFLDYWRLSGHQRVGFLIGQYQP 161

    Q: 3477 HKDIPLGIRAEVAAIYEPPQIGTQNSLELLEDPKAEVVDIEAALGLRKGWIFTDLVSE 3298
    H ++PLGI+A VAAIYEPPQ ++ +E LED + +D + LGL++VGWIFTD +
25  S: 162 HLEVP LGIKATVAAIYEPPQHCRREDGIEFLDKNQKTIDNLEMLGLQRVGWIFTD CWT A 221

    Q: 3297 DTRKGTVRYSRNKDITYFLSSEECITAGDFQNKHPNMCRSLSPDGHFSGKFVTA VAGGPDN 3118
    ++ +GTV Y+R+KD++FLS+EECITA QN+HPN+ S D H+GSKFVT VA+G
    S: 222 NSAEGTVHYTRHKDSFFLSAEBCITAAMLQNHNPITEYSMDRHYGSKFVT VVASGDESM 281

30  Q: 3117 QVHFEGYQVSNQCMALVRDECLLPCKDAPELATPRLAVS 2998
    V+F GYQVSNQC A+V + L P PELA R +S
    S: 282 HVNFHGYQVSNQCAAMVEADILCPTLYTPELAYVRETPLS 321

35  Score = 335 (117.9 bits), Expect = 3.1e-101, Sum P(4) = 3.1e-101
    Identities = 72/215 (33%), Positives = 115/215 (53%), Frame = -1

    Q: 3034 PGAGYAKES--SSEXYVPDVYKDVDFKGFNEITQLARLPVVEYLIIDITTTFPKDPVYTF 2861
    P Y +E+ S E Y+ DV + ++G E+ + RPLPVEYL++D+ PK+P YTF
40  S: 310 PELAYVRETPLSEHYITDVQFSMKNEYGAEMKNGRPLPVEYLLVDVPAGMPKPEPHYTF 369

    Q: 2860 SISQN-----PPIENRDVLGETQDFHSLATYLSQNXSSVFLDTISDFHLLFLVTNEV 2699
    + + F +ENR +G+ Q +L Y S+ ++ FL+ ++FH LL+LVTN+
    S: 370 HVGTSNKSXSAKFVNENRQAIGQLQGGANLIQYSSEFSTNQFLEQATNFHFLLYLVTDQ 429

45  Q: 2698 MPLQDS-ISLLEAVRTRNELAQTWKRS-EQWATIEQLCSTVGQQ-----LPLGHEYGAV 2537
    + + D + L +AV+ ++ A W+ E W + L GG +P +

```


S: 430 VQISDEWMKRLCDAVKAQDRGTAMEWAQECEDWHQLMALAHANGSGNDIPVIPNGDPF 489
 Q: 2536 GGSTHTAT--AAMWACQHCTFMNQPGTGHCEMCSLP 2435
 GS+ + A+W+C HCTF N+ G C MC LP
 5 S: 490 SGSSSGSGRGAVWSCGHCTFQNEAGRQDCSMCGLP 524

The segments of gil2088839 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 163 and SEQ ID NO. 165. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 164 and/or SEQ ID NO. 166 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Endometrial Tumor and to a lesser extent in Soares infant brain 1NIB; Soares_fetal_heart_NbHH19W; Soares_pregnant_uterus_NbHPU; 20 NCI_CGAP_Co3; Soares_testis_NHT; Soares_total_fetus_Nb2HF8_9w; Human Fetal Brain; Human Microvascular Endothelial Cells, fract. A; Activated T-cell(12h)/Thiouridine-re-excision; Soares placenta Nb2HP; Soares fetal liver spleen 1NFLS; Soares_fetal_heart_NbHH19W; Infant brain, Bento Soares; Normal Human Trabecular Bone Cells; Human Eosinophils; Early Stage Human Brain; Colon Tumor 25 II; Activated T-Cell (12hs)/Thiouridine labelledEco; Soares_pregnant_uterus_NbHPU; Human Fetal Kidney; NCI_CGAP_Pr22; Soares_pregnant_uterus_NbHPU; Soares_fetal_liver_spleen_1NFLS_S1; Stratagene hNT neuron (#937233); Stratagene lung carcinoma 937218; Human Pancreas Tumor,

- Reexcision; breast lymph node CDNA library; Soares melanocyte 2NbHM; Human Fetal Kidney, Reexcision; human tonsils; Soares_fetal_heart_NbHH19W; Monocyte activated; Stratagene neuroepithelium NT2RAMI 937234; Nine Week Old Early Stage Human; Soares_testis_NHT; Human Fetal Brain; Amniotic Cells - TNF
- 5 induced; Soares retina N2b4HR; Stratagene HeLa cell s3 937216; Glioblastoma; Human Pituitary, subt IX; T-Cell PHA 16 hrs; NCL_CGAP_Co12; NCL_CGAP_GCB1; NCL_CGAP_Kid3; NCL_CGAP_Brn25; Human Thymus Stromal Cells; Soares breast 2NbHBst; Human Whole Six Week Old Embryo; Macrophage-oxLDL, re-excision; Fetal Heart; Soares_pregnant_uterus_NbHPU;
- 10 Brain frontal cortex; Soares_placenta_8to9weeks_2NbHP8to9W; Soares_fetal_liver_spleen_1NFLS_S1; Smooth muscle, control; Pancreatic Islet; Stratagene muscle 937209; H. hypothalamus, frac A, re-excision; Thyroid Thyroiditis; Soares ovary tumor NbHOT; Messangial cell, frac 1; Osteoclastoma-normalized B; Human Pituitary; Normal Prostate; H. hypothalamus, frac A; Human Tonsil, Lib 3;
- 15 Human 8 Week Whole Embryo, subtracted; H. Striatum Depression, subt; HUMAN TONSILS, FRACTION 2; Human Adult Spleen; Human Aortic Endothelium; Human Cerebellum, subtracted; Human Cardiomyopathy, subtracted; Human Pituitary, subtracted; Early Stage Human Lung, subtracted; Human Quadriceps; Human Soleus; Soares_total_fetus_Nb2HF8_9w; Human adult small intestine, re-excision; Human
- 20 Normal Breast; Messangial cell, frac 2; Soares adult brain N2b4HB55Y; Stratagene fetal retina 937202; B Cell lymphoma; Human Hypothalamus, schizophrenia, re-excision; Human endometrial stromal cells-treated with estradiol; Smooth muscle, IL1b induced; H Female Bladder, Adult; Human Fetal Epithelium (Skin); H. Kidney Cortex, subtracted; Human Stomach, re-excision; HL-60, PMA 4H, re-excision;
- 25 Stratagene neuroepithelium NT2RAMI 937234; Myeloid Progenitor Cell Line; Prostate BPH; H. Lymph node breast Cancer; Brain Frontal Cortex, re-excision;

- Human Prostate; H. Kidney Medulla, re-excision; Human Umbilical Vein, Reexcision; Human Dermal Endothelial Cells, untreated; HMI; Human adult (K. Okubo); NCI_CGAP_AA1; NCI_CGAP_Co2; NCI_CGAP_Ew1; NCI_CGAP_GC4; NCI_CGAP_Lu5; NCI_CGAP_Pr2; NCI_CGAP_Pr3;
- 5 NCI_CGAP_CLL1; NCI_CGAP_Co10; NCI_CGAP_Kid1; NCI_CGAP_Kid6; NCI_CGAP_Pr25; Soares_fetal_heart_NbHH19W; Human Umbilical Vein Endothelial Cells, uninduced; Human Adult Testes, Large Inserts, Reexcision; Stromal cell TF274; Human Hypothalamus, Schizophrenia; STRATAGENE Human skeletal muscle cDNA library, cat. #936215; Stratagene fetal retina 937202; Human
- 10 Ovary; Human umbilical vein endothelial cells, IL-4 induced; Spinal cord; Human Rhabdomyosarcoma; Human adult testis, large inserts; Pancreas Islet Cell Tumor; HM3; Soares_testis_NHT; Soares_total_fetus_Nb2HF8_9w; Human epidermal keratinocyte; Soares_fetal_liver_spleen_INFLS_S1; Colon Carcinoma; Smooth muscle, serum treated; Soares_multiple_sclerosis_2NbHMSP; Soares breast
- 15 3NbHBst; Human fetal heart, Lambda ZAP Express; Human Testes, Reexcision; Human Placenta; Human Neutrophil, Activated; Endothelial-induced; Endothelial cells-control; Human Osteoclastoma; Human Amygdala; Soares_fetal_Jung_NbHL19W; HUMAN B CELL LYMPHOMA; Human Bone Marrow, treated; Bone Marrow Cell Line (RS4, I1); Osteoblasts; Keratinocyte;
- 20 Human 8 Week Whole Embryo; Human Cerebellum and Primary Dendritic Cells, lib 1.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:42 and may have been publicly available prior to conception of
- 25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3839 of SEQ ID NO:42, b is an integer of 15 to 3853, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 33

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Umbilical Vein, Reexcision; Soares placenta Nb2HP; Soares fetal liver spleen 1NFLS and to a lesser extent in Soares infant brain 1NIB; Human Adult Small Intestine; NCI_CGAP_GCB1; Human Liver, normal; Human Fetal Heart; KMH2 cell line; prostate-edited; Human White Adipose; Hepatocellular Tumor, re-excision; H. Kidney Cortex, subtracted; H. Meningima, M1; Spinal Cord, re-excision; NCI_CGAP_Kid6; normalized infant brain cDNA; Human Thymus; Human Brain, Striatum; Monocyte activated, re-excision; L428; Human Hippocampus; Bone Marrow Stromal Cell, untreated; Human Whole Six Week Old Embryo; Smooth muscle, serum induced, re-exc; Macrophage-oxLDL, re-excision; Fetal Heart; Human Substantia Nigra; Human Microvascular Endothelial Cells, fract. A; Monocyte activated and Bone Marrow Cell Line (RS4,11).

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:43 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 542 of SEQ ID NO:43, b is an integer of 15 to 556, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a

5 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 34

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil3411185 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "(AF076240) putative ferredoxin reductase MocF [Rhizobium leguminosarum bv. viciae]". A partial alignment demonstrating the observed homology is shown immediately below.

15 >gi|3411185 (AF076240) putative ferredoxin reductase MocF [Rhizobium
leguminosarum bv. viciae] >sp|085778|085778 PUTATIVE
FERREDOXIN
20 REDUCTASE MOCF.
Length = 421

Plus Strand HSPs:

Score = 317 (111.6 bits), Expect = 6.6e-32, Sum P(2) = 6.6e-32
25 Identities = 91/299 (30%), Positives = 146/299 (48%), Frame = +2

Q: 713 PPSFYVSAQDLPHIENGSGVAULTGKKVQQLDVRDNMVKLNDGSGQITYEKLIATGGTFRS 892
PP YV+ E + +LTG V +D + +V+L+DG + Y++ L+ATG PR
S: 66 PPQ--YVAGP--ARYEEARITILTGVVDITVDROSKLVRLSDGKRFPFYDQLLATGARFRP 122

Q: 893 LSAIDRAGAEVKSRTTLFRKIGDFRSLEKISREVKSITTIIGGGFLGSELACALGRKARAL 1072
+ + +G R + R GD ++ + KS+ ++GGGF+G E+A AR L
S: 123 PAGV--GGDPGLGRICMLRNHGDVAIHEALAPGKSLAVVGGGFIGLETAAT----ARKL 176

Q: 1073 GTEVIQLFPEKGNMKGILPEYLSNWMKEVRREGVVKVMPNAIVQSVGVSSGKLLIKLEDG 1252
G EV+ + + +P ++ E+ R+EGV ++ + S+ + K+ I +G
S: 177 GAEVLLIESSPRVLSRGVPAETAKRVVTERHRQEGVNI LCGEQITSIATENDKVQILFANG 236

Q: 1253 RKVETDHIIVAAVGLPEPNVELAKTGGLEIDSDFGGPRVNAELQARS--NIWVAGDAACFYDI 1429
D +V +G+ PNVELA+ GL ID+ G V+A L+ +I+ AGD F
40

S: 237 TTRLADIVVVGIGVIPNVELAEAAGLLIDN---GIAVDATLRTSDPDIFAAGDCCSFPLS 293
 Q: 1430 KLGRRRV--EHHDHAVVSGRLAGENMTGAAPYWHQSMFWSDLGPDVGYEAIGLVDSSLP 1603
 RRV E +A G L N+ G FWS D ++ + GL D +
 5 S: 294 HYRERRVRLEAWRNAQDQGMLVAANLLGRGLAIASVPWFWSQY-ELTLQIAGLSDGAAT 352
 Q: 1604 TV 1609
 TV
 S: 353 TV 354
 10

The segment of gil3411185 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 167. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein.

15 Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 168 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a
 20 sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Primary Dendritic Cells, lib 1 and to a lesser extent in Soares ovary tumor NbHOT; NCL_CGAP_Kid3; Human Testes; Soares_parathyroid_tumor_NbHPA; Human adult small intestine, re-excision; Human
 25 Adult Small Intestine; Human heart cDNA (YNakamura); T cell helper II; Soares fetal liver spleen INFLS; Human Cardiomyopathy, subtracted; Stratagene colon (#937204); H. Kidney Medulla, re-excision; L428; Human Heart; Soares_fetal_liver_spleen_INFLS_S1; Human Liver, normal; Endothelial-induced; Human Osteoclastoma; Stratagene muscle 937209; T Cell helper I; Bone Marrow
 30 Cell Line (RS4,11); Activated T-cell(12h)/Thiouridine-re-excision; Nine Week Old

- Early Stage Human; Soares placenta Nb2HP; Soares infant brain 1N1B; Gessler Wilms tumor; Soares_testis_NHT; Soares_NFL_T_GBC_S1; Stratagene HeLa cell s3 937216; Human B Cell 8866; H. Striatum Depression, subtracted; Human Adult Spleen; Human OB MG63 treated (10 nM E2) fraction I; Human Thyroid; Raji Cells, cyclohexamide treated; NCI_CGAP_AA1; NCI_CGAP_Br2; NCI_CGAP_Sch1; HEL cell line; Glioblastoma; LNCAP prostate cell line; Myeloid Progenitor Cell Line; Human Fetal Kidney; Human Uterine Cancer; Human Placenta (re-excision); Epithelial-TNF α and INF induced; Fetal Liver, subtraction II; Human T-Cell Lymphoma; H Macrophage (GM-CSF treated), re-excision; NCI_CGAP_GCB1;
- 10 Normal colon; Human Synovial Sarcoma; Primary Dendritic cells, frac 2; Soares_NhHMPu_S1; Human Microvascular Endothelial Cells, fract. A; Smooth muscle, control; Monocyte activated; HUMAN B CELL LYMPHOMA; Human Endometrial Tumor and Human 8 Week Whole Embryo.

- Many polynucleotide sequences, such as EST sequences, are publicly
- 15 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:44 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
- 20 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2136 of SEQ ID NO:44, b is an integer of 15 to 2150, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 35

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil4103490 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "(AF024519) glucocorticoid-induced leucine zipper GILZ protein [Mus musculus]." This novel murine protein is thought to protect T lymphocytes from TCR/CD3 activated cell death. A partial alignment demonstrating the observed homology is shown immediately below.

```

>gi|4103490 (AF024519) glucocorticoid-induced leucine zipper GILZ protein
[Mus musculus] >sp|G4103490|G4103490 GLUCOCORTICOID-INDUCED LEUCINE
ZIPPER GILZ PROTEIN.
Length = 137

Plus Strand HSPs:

Score = 584 (205.6 bits), Expect = 4.6e-56, P = 4.6e-56
Identities = 119/137 (86%), Positives = 124/137 (90%), Frame = +1

Q: 271 MNTEMYQTPMEVAVYQLHNXXXXXXXXLLGGDVVSVKLDNSASGASVVAIDNKNIEQAMD 450
MNTMYQTPMEVAVYQLHN LLGGDVVSVKLDNSASGASVVAIDNKNIEQAMD 450
S: 1 MNTEMYQTPMEVAVYQLHNFSTSFSSLLGGDVVSVKLDNSASGASVVAIDNKNIEQAMD 60

Q: 451 VKNHLMYAVREEVEILKEQIRELVKNSQLERENTLLKTASPEQLEKFQSCLSPEEPAP 630
VKNHLMYAVREEVE+LKEQIREL+EKNSQLERENTLLKTASPEQLEKFQSLPEEPAP 630
S: 61 VKNHLMYAVREEVEVLKEQIRELLEKNSQLERENTLLKTASPEQLEKFQSRSLPEEPAP 120

Q: 631 ESPQVPE---APGGSAAV 672
E+P+ PE APGGSAAV
S: 121 EAPETPETPEAPGGSAAV 137

```

The segment of gil4103490 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 169. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities.

Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the
5 amino acid sequence set out in the sequence listing as SEQ ID NO. 170 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

When tested against sensory neuron cell lines, supernatants removed from cells containing this gene activated the EGR1 assay. Thus, it is likely that this gene
10 activates sensory neuron cells through a signal transduction pathway. Early growth response 1 (EGR1) is a promoter associated with certain genes that induces various tissues and cell types upon activation, leading the cells to undergo differentiation and proliferation.

It has been discovered that this gene is expressed primarily in the following
15 tissues/cDNA libraries: Soares ovary tumor NbHOT; Soares_fetal_heart_NbHH19W and to a lesser extent in Human Neutrophil, Activated; Human Neutrophil; Human Thymus; Stratagene lung (#937210); Human Bone Marrow, treated; Soares_multiple_sclerosis_2NbHMSP; Human adult small intestine, re-excision; Human Adult Small Intestine; Rejected Kidney, lib 4; Resting T-Cell Library, II;
20 Colon Normal II; Neutrophils control, re-excision; Human Cerebellum; Soares infant brain 1N1B; Human osteoarthritic, fraction II; Human osteoarthritis, fraction I; Soares retina N2b4HR; Human Chronic Synovitis; Human Adrenal Gland Tumor; normalized infant brain cDNA; Human T-Cell Lymphoma; Human Adult Spleen; Soares_senescent_fibroblasts_NbHSF; H. Meningioma, M1; Human Uterine Cancer;
25 NCI_CGAP_CLL1; Soares_testis_NHT; Fetal Heart; NCI_CGAP_Lu5; NCI_CGAP_GCB1; Soares breast 3NbHBst; NCI_CGAP_Co3; Normal colon;

- Human Synovial Sarcoma; Human Adult Pulmonary, re-excision; Keratinocyte; T cell helper II; Human Osteoarthritic Cartilage Fraction IV;
- Soares_total_fetus_Nb2HF8_9w; Human Prostate BPH, re-excision; Human White Fat; Dermatofibrosarcoma Protuberance; LNCAP untreated; Human Adult
- 5 Pulmonary; H. Atrophic Endometrium; Human Quadriceps; Human T-cell lymphoma, re-excision; Human Tonsils; Lib 2; Alzheimers, spongy change; Human Adipose Tissue, re-excision; Prostate BPH; Brain Frontal Cortex, re-excision; Human Infant Brain; Human Bone Marrow, re-excision; Human Hypothalamus, Schizophrenia; Human Pancreas Tumor, Reexcision; Olfactory epithelium, nasal cavity;
- 10 Soares_pregnant_uterus_NbHPU; Ulcerative Colitis; Fetal Liver, subtraction II; Soares_NFL_T_GBC_S1; Human Gall Bladder; Colon Tumor; Soares_pregnant_uterus_NbHPU; Stratagene colon (#937204); Soares_fetal_heart_NbHH19W; Smooth muscle, serum treated; Dendritic cells, pooled; NCI_CGAP_Pr1; NCI_CGAP_GCB1; Human Fetal Kidney, Reexcision;
- 15 Human Placenta; human tonsils; CD34 depleted Buffy Coat (Cord Blood), re-excision; Smooth muscle, control; NCI_CGAP_CLL1; Spleen, Chronic lymphocytic leukemia; Bone Marrow Cell Line (RS4,11); Activated T-cell(12h)/Thiouridine-re-excision; Soares placenta Nb2HP; Soares fetal liver spleen 1NFLS; H. Leukocytes, normalized cot > 500A; Human Fetal Kidney; Human Normal Cartilage Fraction III;
- 20 Human Lung Cancer, subtracted; Human Osteoarthritic Cartilage Fraction III; Human Gastrocnemius; Human Placenta, subtracted; Bone marrow stroma, treated; Human Normal Cartilage Fraction II; Human Leukocytes; H. Adipose Tissue; Human White Adipose; Human Pituitary, subtracted; Human Neutrophils, Activated, re-excision; Smooth Muscle Serum Treated, Norm; Supt Cells, cyclohexamide treated; Smooth
- 25 muscle, control, re-excision; Smooth muscle-ILb induced; Invasive poorly differentiated lung adenocarcinoma, metastatic; Human Thyroid; Early Stage Human

- Lung, subtracted; Soares_fetal_heart_NbHH19W; Soares_pregnant_uterus_NbHPU;
 Raji Cells, cyclohexamide treated; Human Normal Breast;
 Soares_multiple_sclerosis_2NbHMSP; Human Lung Cancer, re-excision; B Cell
 lymphoma; Human Epididymus; Human Hypothalamus, schizophrenia, re-excision;
- 5 Human Synovium; Human Prostate Cancer, Stage C fraction; pBMC stimulated w/
 poly I/C; Smooth muscle, IL1b induced; Human endometrial stromal cells-treated
 with progesterone; LNCAP prostate cell line; Human Amygdala, re-excision; Human
 Osteosarcoma; Human Colon, re-excision; Soares_parathyroid_tumor_NbHPPA;
 Stratagene HeLa cell s3 937216; Apoptotic T-cell; Human Activated T-Cells; Human
- 10 Pancreas Tumor; Human Heart; NCI_CGAP_Kid3; Soares_pregnant_uterus_NbHPU;
 Liver, Hepatoma; Human Adipose; NCI_CGAP_Kid6; Human Testes Tumor, re-
 excision; Hemangiopericytoma; Bone Marrow Stromal Cell, untreated; Soares breast
 2NbHBst; Barstead spleen HPLRB2; Jia bone marrow stroma;
 Soares_fetal_heart_NbHH19W; Soares_fetal_liver_spleen_INFLS_S1; Stratagene
- 15 hNT neuron (#937233); Smooth muscle, serum induced, re-exc; Pancreas Islet Cell
 Tumor; Human adult lung 3' directed Mbol cDNA; NCI_CGAP_Pr2;
 NCI_CGAP_Pr22; Soares_pregnant_uterus_NbHPU;
 Soares_total_fetus_Nb2HF8_9w; Soares_senescent_fibroblasts_NbHSF; b4HB3MA;
 Stratagene endothelial cell 937223; 12 Week Old Early Stage Human; Infant brain,
- 20 LLNL array of Dr. M. Soares 1NIB; Neutrophils IL-1 and LPS induced;
 NCI_CGAP_Kid5; Human Eosinophils; breast lymph node CDNA library; Human
 Testes Tumor; Stratagene endothelial cell 937223; Colon Tumor II; Human adult
 (K.Okubo); NCI_CGAP_Br3; NCI_CGAP_Lu1; NCI_CGAP_Pr2; NCI_CGAP_Pr6;
 NCI_CGAP_Pr9; NCI_CGAP_Pr22; NCI_CGAP_Pr23; Colon Normal III; Human
- 25 Amygdala; Human Microvascular Endothelial Cells, fract. A; HUMAN B CELL
 LYMPHOMA; Human aorta polyA+ (TFujiwara); Human fetal heart, Lambda ZAP

Express; NCI_CGAP_Brn23; Hodgkin's Lymphoma II; Nine Week Old Early Stage Human and Primary Dendritic Cells, lib 1.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 94 as residues: Val-80 to Leu-92, Ser-98 to Lys-104,

5 Pro-111 to Pro-122. Polynucleotides encoding said polypeptides are also provided.

Given the homology of this protein to the murine leucine zipper protein, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:45 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
25 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 1994 of SEQ ID NO:45, b is an integer of 15 to 2008, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 36

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares melanocyte 2NbHM and to a lesser extent in Soares breast 3NbHBst; Soares_pregnant_uterus_NbHPU; Stratagene ovarian cancer (#937219); Soares_NbHMPu_S1; Soares ovary tumor NbHOT; NCI_CGAP_Co8; Soares_NFL_T_GBC_S1; Soares_total_fetus_Nb2HF8_9w; Soares_multiple_sclerosis_2NbHMSP; Smooth muscle, control; H. hypothalamus, frac A, re-excision; Human adult (K.Okubo); Human fetal heart, Lambda ZAP Express; NCI_CGAP_Lu5; NCI_CGAP_Kid3; Soares_testis_NHT; HUMAN
15 STOMACH; Human Tonsils, Lib 2; Human Prostate; Stratagene lung (#937210) and Soares infant brain 1NIB.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 95 as residues: Arg-15 to Ser-27, Ser-29 to Tyr-41, Thr-55 to Phe-62. Polynucleotides encoding said polypeptides are also provided.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:46 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
25 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 1460 of SEQ ID NO:46, b is an integer of 15 to 1474, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:46, and where b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 37

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Primary Breast Cancer Reexcision; Human Cerebellum and to a lesser extent in Soares ovary tumor NbHOT; Activated T-Cells,
- 10 Ohrs, subtracted; Soares_pregnant_uterus_NbHPU; Nine Week Old Early Stage Human; normalized infant brain cDNA; NCI_CGAP_HSC2; Olfactory epithelium,nasalcavity; Rejected Kidney, lib 4; Human adult testis, large inserts; NTERA2, control; breast lymph node CDNA library; Soares melanocyte 2NbHM; NCI_CGAP_GCB1; NCI_CGAP_Pr22; Bone Marrow Cell Line (RS4,11);
- 15 Osteoblasts; Human 8 Week Whole Embryo; T cell helper II; Soares infant brain INIB; normalized infant brain cDNA; Infant brain, Bento Soares; Soares_testis_NHT; Thyroid Tumour; Stomach Tumour; CD34+ cell, I, frac II; Human OB HOS treated (1 nM E2) fraction I; Human Fetal Brain, random primed; LNCAP untreated; Breast Lymph node cDNA library; Resting T-Cell, re-excision;
- 20 human corpus colosum; Human endometrial stromal cells-treated with progesterone; Human endometrial stromal cells; Jurkat T-Cell, S phase; Healing groin wound, 6.5 hours post incision; L428; Human Osteoblasts II; Human Pancreas Tumor; NCI_CGAP_Lu26; Human Activated T-Cells, re-excision; NCI_CGAP_Kid3; NCI_CGAP_Brn25; Human Testes Tumor, re-excision; CHME Cell Line,treated 5
- 25 hrs; Hepatocellular Tumor, re-excision; Pancreas Islet Cell Tumor; PC3 Prostate cell line; Colon Tumor; Smooth muscle, serum treated; Human Placenta;

NCI_CGAP_Br2; NCI_CGAP_GC4; NCI_CGAP_Kid3; NCI_CGAP_Kid6;
 NCI_CGAP_Pr12; Colon Tumor II; Human Synovial Sarcoma; Human fetal lung; 12
 Week Early Stage Human II, Reexcision; NCI_CGAP_Pr28; NCI_CGAP_Brn25;
 NCI_CGAP_Kid11; Soares_fetal_heart_NbHH19W;

- 5 Soares_senescent_fibroblasts_NbHSF; NCI_CGAP_Pr11; NCI_CGAP_Pr23; Colon
 Normal III; Monocyte activated; Human Bone Marrow, treated; H. Frontal
 cortex,epileptic,re-excision and Keratinocyte.

- Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are
 10 related to SEQ ID NO:47 and may have been publicly available prior to conception of
 the present invention. Preferably, such related polynucleotides are specifically
 excluded from the scope of the present invention. To list every related sequence
 would be cumbersome. Accordingly, preferably excluded from the present invention
 are one or more polynucleotides comprising a nucleotide sequence described by the
 15 general formula of a-b, where a is any integer between 1 to 1879 of SEQ ID NO:47, b
 is an integer of 15 to 1893, where both a and b correspond to the positions of
 nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a
 + 14.

20 FEATURES OF PROTEIN ENCODED BY GENE NO: 38

- It has been discovered that this gene is expressed primarily in the following
 tissues/cDNA libraries: Human Endometrial Tumor; Soares infant brain 1NIB and to
 a lesser extent in NCI_CGAP_GCBI; Human 8 Week Whole Embryo;
 Soares_senescent_fibroblasts_NbHSF; Human Adrenal Gland Tumor;
 25 NCI_CGAP_Br2; NCI_CGAP_Lu5; NCI_CGAP_Kid5; CHME Cell Line,untreated;
 Soares_total_fetus_Nb2HF8_9w; Soares_parathyroid_tumor_NbHPA; normalized

- infant brain cDNA; Human Fetal Kidney, Reexcision; Soares fetal liver spleen
 INFLS; Larynx carcinoma IV; Pharynx carcinoma; Human Colon; Amniotic Cells -
 TNF induced; NTERA2 teratocarcinoma cell line+retinoic acid (14 days);
 Soares_multiple_sclerosis_2NbHMSP; Soares_placenta_8to9weeks_2NbHP8to9W;
- 5 H. Whole Brain #2, re-excision; HEL cell line; Human endometrial stromal cells-
 treated with estradiol; Glioblastoma; Jurkat T-cell G1 phase; Human Infant Brain;
 Human Uterine Cancer; Human Ovary; Human Rhabdomyosarcoma; CHME Cell
 Line, treated 5 hrs; HTC DL1; Human retina cDNA Tsp509I-cleaved sublibrary;
 NCI_CGAP_AA1; NCI_CGAP_Co3; NCI_CGAP_GC2; NCI_CGAP_Ov2;
- 10 NTERA2, control; Human Liver, normal; HTC DL1; Liver HepG2 cell line.;
 Soares_fetal_liver_spleen_INFLS_S1; Soares_senescent_fibroblasts_NbHSF;
 Stratagene cDNA library Human heart, cat#936208; Human Fetal Lung III; Human
 Testes, Reexcision; human tonsils; Activated T-Cell (12hs)/Thiouridine labelled Eco;
 Human Amygdala; HUMAN B CELL LYMPHOMA; Human Testes; Activated T-
- 15 cell(12h)/Thiouridine-re-excision; Soares_pregnant_uterus_NbHPU and T cell helper
 II.

Many polynucleotide sequences, such as EST sequences, are publicly
 available and accessible through sequence databases. Some of these sequences are
 related to SEQ ID NO:48 and may have been publicly available prior to conception of
 20 the present invention. Preferably, such related polynucleotides are specifically
 excluded from the scope of the present invention. To list every related sequence
 would be cumbersome. Accordingly, preferably excluded from the present invention
 are one or more polynucleotides comprising a nucleotide sequence described by the
 general formula of a-b, where a is any integer between 1 to 2404 of SEQ ID NO:48, b
 25 is an integer of 15 to 2418, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 39

5 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human adult testis, large inserts; Human (HCC) cell line liver (mouse) metastasis, remake; Human Adult Testes, Large Inserts, Reexcision; CD34 positive cells (Cord Blood).

Many polynucleotide sequences, such as EST sequences, are publicly
10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:49 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
15 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1145 of SEQ ID NO:49, b is an integer of 15 to 1159, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 40

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.
25 gil182221 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "ORF 3 [Homo

sapiens)". A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >gi|182221 ORF 3 [Homo sapiens] >pir|E41925|E41925 hypothetical protein 3
      -
          human >sp|Q14270|Q14270 ORF 3.
          Length = 143

      Plus Strand HSPs:

10      Score = 188 (66.2 bits), Expect = 4.2e-17, Sum P(2) = 4.2e-17
      Identities = 42/107 (39%), Positives = 58/107 (54%), Frame = +1

15      Q: 2020 LYMNLNHISKWLTVIKVVTTRKMQIKTTLQYHCTPTSTV*KKKKAENIKYW*QCGANRTLF 2199
          +Y H+ K + + + R+MQIKTT++YH TP V +K N + W CG TL
      S: 10 IYAANRHMKKCSSSLAI--REMGIKTTMRYHLTPVRMVIIRKSG--NDRCWRCGEIGTLL 66

      Q: 2200 Q*WQVCKVAQTLNKPVWHYTVLNLNTRIVYDPGMPPLGTNNNAYM-CC 2340
          W CK+ Q LNK VW + + +L I +DP +PLLG Y CC
20      S: 67 HCWWDCKLVQPLNKTWVRF-LRDLQLEIPFDPATPFLGIYPKDYKSCC 113

```

The segment of gi182221 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 171. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 172 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares fetal liver spleen 1NFLS and to a lesser extent in Human 8 Week Whole Embryo; Soares placenta Nb2HP; Human Chondrosarcoma; Hodgkin's Lymphoma II; Nine Week Old Early Stage Human;

- Soares_parathyroid_tumor_NbHPA; Human Testes, Reexcision; Soares retina N2b4HR; Temporal cortex-Alzheimer, subtracted; Soares_testis_NHT;
- Soares_parathyroid_tumor_NbHPA; NCI_CGAP_GCB1; Frontal lobe,dementia,re-excision; Human Thyroid; Human T-cell lymphoma,re-excision; T-Cell PHA 16 hrs;
- 5 Human fetal heart, Lambda ZAP Express; NCI_CGAP_Lei2;
- Soares_NFL_T_GBC_S1; Stromal cell TF274; Soares adult brain N2b5HB55Y;
- Human Adrenal Gland Tumor; Pancreas Islet Cell Tumor; Human Osteoclastoma; H. Frontal cortex,epileptic,re-excision; Soares_pregnant_uterus_NbHPU; Human Lung Cancer, subtracted; Thyroid Normal (SDCA2 No); H. Striatum Depression, subt;
- 10 Activated T-cells; Papillary serous cystic neoplasm of low malignant potential (ovary); Soares_pregnant_uterus_NbHPU; Human Normal Breast; B Cell lymphoma; Human Hypothalamus,schizophrenia, re-excision; Hepatocellular Tumor; pBMC stimulated w/ poly I/C; H Female Bladder, Adult; H. Kidney Cortex, subtracted; Healing groin wound, 7.5 hours post incision; Pancreas normal PCA4 No; Synovial
- 15 hypoxia; Soares_pregnant_uterus_NbHPU; Spinal Cord, re-excision; Human Prostate; Soares_parathyroid_tumor_NbHPA; Human Bone Marrow, re-excision; Gessler Wilms tumor; Human promyelocyte; Human fetal brain (TFujiwara); NCI_CGAP_HN4; NCI_CGAP_Pr12; Soares_pregnant_uterus_NbHPU; Stratagene fibroblast (#937212); Stratagene hNT neuron (#937233); normalized infant brain
- 20 cDNA; Human Umbilical Vein Endothelial Cells, uninduced; Human Pancreas Tumor; Human Hippocampus; Synovial Fibroblasts (control); Human Whole Six Week Old Embryo; NTERA2, control; NCI_CGAP_GCB1; Hepatocellular Tumor, re-excision; NCI_CGAP_GCB1; Soares breast 3NbHBst; NCI_CGAP_Pr12; NCI_CGAP_Thy1; Human Amygdala; Human Bone Marrow, treated; Human Testes;
- 25 Keratinocyte; Soares_fetal_heart_NbHH19W; Stratagene fibroblast (#937212); T cell helper II and Soares_multiple_sclerosis_2NbHMSP.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 99 as residues: Ser-31 to Lys-39. Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:50 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2778 of SEQ ID NO:50, b is an integer of 15 to 2792, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.

15

FEATURES OF PROTEIN ENCODED BY GENE NO: 41

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil3170458 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "(AF053003) diphthamide biosynthesis protein-2; DPH2 [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

25

```
>gi|3170458 (AF053003) diphthamide biosynthesis protein-2; DPH2 [Homo sapiens]
      >sp|O60623|O60623 DIPHTHAMIDE BIOSYNTHESIS PROTEIN-2.
      Length = 489
```

Minus Strand HSPs:

Score = 1433 (504.4 bits), Expect = 5.1e-146, P = 5.1e-146
Identities = 275/336 (81%), Positives = 275/336 (81%), Frame = -1

5 Q: 1888 EPACAHAEALATLLRPRYDLLVSSPAFFQPVGSLSPPEMPLERFGRFRFLAPGRRL EE 1709
EPACAHAEALALA LLRPRYDLLVSSPAFFQPVGSLSPPEMP ERFGRRFLAPGRRL EE
S: 154 EPACAHAEALAXLLRPRYDLLVSSPAFFQPVGSLSPPEMPXERFGRFRFLAPGRRL EE 213

10 Q: 1708 YGAFYVGGSKAXXXXXXXXXXXXXXGWAQGPFFSSCCPDGTGKTQXXXXXXXXXXXXX 1529
YGAFYVGGSKA GWAPGPFFSSCCPDGTGKTQ
S: 214 YGAFYVGGSKASDPDLDPDLRLGWAQGPFFSSCCPDGTGKTQDEGARAGGLRARRR Y 273

15 Q: 1528 XXXXXXXXVVGLLAGT LGVAQHREALAHLRNLQAAGKRYVLALGRPTAKLANFPEV 1349
VVGLLAGT GVAQHREALAHLRNLQAAGKR YVLALGRPTAKLANFPEV
S: 274 XVERARDARVVGLLAGTXGVAQHREALAHLRNLQAAGKRSYVLALGRPTAKLANFPEV 333

20 Q: 1348 DVEVLLACPLGALAPQLSGSFFQPI LAPCELEAACNPAPWPPGLAPHLTHYADLLPGSPF 1169
DVEVLLACPLGALAPQLSGSFFQPI LAPCELEAACNPAPWPPGLAPHLTHYADLLPGSPF
S: 334 DVEVLLACPLGALAPQLSGSFFQPI LAPCELEAACNPAPWPPGLAPHLTHYADLLPGSPF 393

25 Q: 1168 HVALPPESELWETPDVSLITGDLRPPPAWKSSNDHGSLALTPRPQXXXXXXXXXXXXX 989
HVALPPESELWETPDVSLITGDLRPPPAWKSSNDHGSLAL PRPQ
S: 394 HVALPPESELWETPDVSLITGDLRPPPAWKSSNDHGSLALNRPQLXLAESSPAVSFLS 453

Q: 988 XXXWQGLEPRLGQTPVTEAVSGRRGIAIAYEDEGSG 881
WQGLEPRLGQTPVTEAVSGRRGIAIAYEDEGSG
S: 454 SRSWQGLEPRLGQTPVTEAVSGRRGIAIAYEDEGSG 489

30 The segment of gil3170458 that is shown as "S" above is set out in the
sequence listing as SEQ ID NO. 173. Based on the structural similarity, these
homologous polypeptides are expected to share at least some biological activities.
Such activities are known in the art, some of which are described elsewhere herein.
Assays for determining such activities are also known in the art, some of which have
35 been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the
amino acid sequence set out in the sequence listing as SEQ ID NO. 174 which
corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a
sequence by the computer are, of course, removed).

40 It has been discovered that this gene is expressed primarily in the following
tissues/cDNA libraries: Activated T-cell(12h)/Thiouridine-re-excision and to a lesser

- extent in NCI_CGAP_GCB1; Soares fetal liver spleen 1NFLS; Soares infant brain 1N1B; Pharynx carcinoma; Human Testes Tumor; Activated T-Cell (12hs)/Thiouridine labelledEco; Soares_fetal_heart_NbHH19W; NCI_CGAP_Co3; Soares_fetal_Jung_NbHL19W; Soares_testis_NHT; Soares ovary tumor NbHOT;
- 5 Soares_total_fetus_Nb2HF8_9w; Soares_testis_NHT; Raji cells, cyclohexamide treated, subtracted; Human B Cell 8866; Human epithelioid sarcoma; Human Aortic Endothelium; Human Primary Breast Cancer, re-excision; L428; Human Fetal Kidney; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Human Chondrosarcoma; Human Thymus; Human Testes Tumor, re-excision; Human
- 10 Adrenal Gland Tumor; human tonsils; Endothelial-induced; Human Adult Pulmonary, re-excision; Human Amygdala; Human Microvascular Endothelial Cells, fract. A; Human Bone Marrow, treated; NCI_CGAP_GC5; NCI_CGAP_Lu1; NCI_CGAP_Co12; NCI_CGAP_Pr12; NCI_CGAP_Bm23; Soares_fetal_heart_NbHH19W and Stratagene NT2 neuronal precursor 937230.
- 15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:51 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
- 20 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1907 of SEQ ID NO:51, b is an integer of 15 to 1921, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a
- 25 + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 42

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

- 5 gi|296560 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "ORF YBR1729 [*Saccharomyces cerevisiae*]". A partial alignment demonstrating the observed homology is shown immediately below.

```

10  >gi|296560 ORF YBR1729 [Saccharomyces cerevisiae] >gi|536689 ORF YBR261c
    [Saccharomyces cerevisiae] >pir|S32963|S32963 hypothetical
    protein
        YBR261c - yeast (Saccharomyces cerevisiae)
        Length = 232
15
    Plus Strand HSPs:

    Score = 166 (58.4 bits), Expect = 3.7e-10, P = 3.7e-10
    Identities = 38/80 (47%), Positives = 46/80 (57%), Frame = +3
20
    Q:  627 GHLTDQHLAEFLRRCKGSLRPNGLIIVIKDNMAQEGVI-LDDVDSSVCRDLDVVRIICSA 803
        GHL D L FL+RC L+PNG IV+K+N D+ DSSV R R+I A
    S:  143 GHLFDAELVAFLEKRCIVGLQPNGTIVVKENNTPTDITDDFDDETSSVTRSDAKFRQIFEEA 202

25  Q:  804 GLSLLAEERQENLPDEIYHV 863
        GL L+A ERQ LP E+Y V
    S:  203 GLKLIASERQRLPRELYPV 222
  
```

- The segment of gi|296560 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 175. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

- 35 Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 176 which

corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human adult testis, large inserts; Human Fetal Kidney,
- 5 Reexcision; Activated T-cell(12h)/Thiouridine-re-excision and to a lesser extent in Stratagene placenta (#937225); Soares_fetal_heart_NbHH19W; Human Rhabdomyosarcoma; Human Thymus; Bone Marrow Stromal Cell, untreated; Soares_pregnant_uterus_NbHPU; Normal Human Trabecular Bone Cells; NCI_CGAP_GCB1; Osteoblasts; Human Cerebellum; Tongue Tumour; LNCAP +
 - 10 30nM R1881; LNCAP + 0.3nM R1881; Human Pituitary, re-excision; Human (Caco-2) cell line, adenocarcinoma, colon, remake; stomach cancer (human); Human Neutrophils, Activated, re-excision; Smooth muscle-ILb induced; H. Epididymus, caput & corpus; Human Primary Breast Cancer; Human Liver; Apoptotic T-cell, re-excision; Human endometrial stromal cells-treated with estradiol; human corpus
 - 15 colosum; Salivary Gland, Lib 2; LNCAP prostate cell line; Human endometrial stromal cells; Human Prostate; Breast Cancer Cell line, angiogenic; KMH2; Human Thymus; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Human Ovary; Human Activated Monocytes; Soares_placenta_8to9weeks_2NbHP8to9W; Human Fetal Brain; Human Adrenal Gland Tumor; Human Whole Six Week Old Embryo;
 - 20 CHME Cell Line,untreated; H Macrophage (GM-CSF treated), re-excision; Soares melanocyte 2NbHM; Human colorectal cancer; Liver HepG2 cell line.; Soares_total_fetus_Nb2HF8_9w; Soares_senescent_fibroblasts_NbHSF; Bone marrow; human tonsils; Endothelial-induced; Endothelial cells-control; Anergic T-cell; Stratagene NT2 neuronal precursor 937230; neutrophils control; Human fetal
 - 25 heart, Lambda ZAP Express; NCI_CGAP_Pr7; NCI_CGAP_Co10; NCI_CGAP_GCB0; Keratinocyte; T cell helper II and Soares infant brain 1NIB.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 101 as residues: Gly-80 to Gly-88. Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:52 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1480 of SEQ ID NO:52, b is an integer of 15 to 1494, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 43

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain 1N1B and to a lesser extent in Soares_testis_NHT; Soares_fetal_heart_NbHH19W; Human Uterine Cancer; NCI_CGAP_Co3; Soares_NFL_T_GBC_S1; normalized infant brain cDNA and Human Fetal Heart.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:53 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

- would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1686 of SEQ ID NO:53, b is an integer of 15 to 1700, where both a and b correspond to the positions of
- 5 nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 44

- It has been discovered that this gene is expressed primarily in the following
- 10 tissues/cDNA libraries: Human Placenta; Human Testes and to a lesser extent in Soares adult brain N2b5HB55Y; Soares_NbHFB; LNCAP prostate cell line; Human Uterine Cancer; Human Adult Testes, Large Inserts, Reexcision; NCI_CGAP_GCB1; Human Microvascular Endothelial Cells, fract. A; Healing groin wound - zero hr post-incision (control); Stratagene pancreas (#937208); NCI_CGAP_Co8;
- 15 Hepatocellular Tumor, re-excision; 12 Week Old Early Stage Human, II; Soares_fetal_lung_NbHL19W; Soares_parathyroid_tumor_NbHPA; Fetal Liver, subtraction II; Smooth muscle, serum induced, re-exc; Colon Tumor II; Human Testes, Reexcision; NCI_CGAP_Pr1; Soares_placenta_8to9weeks_2NbHP8to9W; Human Bone Marrow, treated; Hodgkin's Lymphoma II; Human Microvascular
- 20 Endothelial Cells, fract. B; Human OB HOS treated (1 nM E2) fraction I; Human Colon, subtraction; LNCAP untreated; Human OB HOS control fraction I; Human OB HOS treated (10 nM E2) fraction I; Human Aortic Endothelium; Human Fetal Bone; human colon cancer; Human Primary Breast Cancer; Amniotic Cells - TNF induced; Cem cells cyclohexamide treated; NTERA2 teratocarcinoma cell
- 25 line+retinoic acid (14 days); HEL cell line; Human Epididymus; Stratagene endothelial cell 937223; Human endometrial stromal cells-treated with estradiol;

- HM1; NCI_CGAP_GC4; NCI_CGAP_HN4; NCI_CGAP_Ov8; NCI_CGAP_GCB1; NCI_CGAP_Kid3; NCI_CGAP_Brn25; Hepatocellular Tumor; Human Prostate Cancer, Stage C fraction; Breast Cancer cell line, MDA 36; Smooth muscle, IL1b induced; Human Stomach, re-excision; Human endometrial stromal cells-treated with
- 5 progesterone; HL-60, PMA 4H, re-excision; Stratagene pancreas (#937208); Stratagene endothelial cell 937223; Human endometrial stromal cells; Human Colon, re-excision; Spleen metastatic melanoma; Human Dermal Endothelial Cells, untreated; Apoptotic T-cell; Stromal cell TF274; Soares_fetal_heart_NbHH19W; Human umbilical vein endothelial cells, IL-4 induced; Human Rhabdomyosarcoma; Human
- 10 Thymus; Hemangiopericytoma; Macrophage-oxLDL, re-excision; Smooth muscle, serum treated; Human Testes Tumor; Human placenta cDNA (TFujiwara); Soares_testis_NHT; Soares_fetal_heart_NbHH19W; Soares_total_fetus_Nb2HF8_9w; Soares_senescent_fibroblasts_NbHSF; 12 Week Early Stage Human II, Reexcision; Endothelial-induced; NCI_CGAP_Br3;
- 15 NCI_CGAP_Ew1; NCI_CGAP_Lu1; NCI_CGAP_Kid1; NCI_CGAP_Kid5; NCI_CGAP_Pr21; NCI_CGAP_Br1.1; Human Primary Breast Cancer Reexcision; Human Osteoclastoma; Human Amygdala; NCI_CGAP_Ov31; T Cell helper I; Keratinocyte and Soares fetal liver spleen INFLS.

- Many polynucleotide sequences, such as EST sequences, are publicly
- 20 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:54 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
- 25 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1163 of SEQ ID NO:54, b

is an integer of 15 to 1177, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 45

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gnllPIDe1254905 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "(AJ002308) synaptogyrin 2 [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

15  >gnl|PID|e1254905 (AJ002308) synaptogyrin 2 [Homo sapiens]
    >sp|O43760|O43760
        SYNAPTOGYRIN 2.
        Length = 224

20      Plus Strand HSPs:

        Score = 1081 (380.5 bits), Expect = 9.1e-109, P = 9.1e-109
        Identities = 205/224 (91%), Positives = 205/224 (91%), Frame = +1

25  Q:   49 MESXXXXXXXXXXSFDLRRFLTQPQVVARAVCLVFLALIVFSCIYEGEGYSNAHESKQMYCV 228
        MES          SFDLRRFLTQPQVVARAVCLVFLALIVFSCIYEGEGYSNAHESKQMYCV
        S:   1 MESGAYGAAGAGGSFDLRRFLTQPQVVARAVCLVFLALIVFSCIYEGEGYSNAHESKQMYCV 60

30  Q:   229 FNRNEDACRYGSAIGVLAFLASAFFLVVDAYFPQISNATDRKKYLVIGDLLFSALWTFPLWF 408
        FNRNEDACRYGSAIGVLAFLASAFFLVVDAYFPQISNATDRKKYLVIGDLLFSALWTFPLWF
        S:   61 FNRNEDACRYGSAIGVLAFLASAFFLVVDAYFPQISNATDRKKYLVIGDLLFSALWTFPLWF 120

35  Q:   409 VGFCELTNQWAVTNPKXVLVGADSVRAAITXXXXXXXXXWGLASLAYQRYKAGVDDFIQN 588
        VGFCELTNQWAVTNPK VLVGADSVRAAIT          WGLASLAYQRYKAGVDDFIQN
        S:   121 VGFCELTNQWAVTNPKDLVLVGADSVRAAITPSFSPISWGLASLAYQRYKAGVDDFIQN 180

        Q:   589 YVDPTDPNNTAYASYPGASVDNYQQPPPTQNAETTEGYQPPPPY 720
        YVDPTDPNNTAYASYPGASVDNYQQPPPTQNAETTEGYQPPPPY
        S:   181 YVDPTDPNNTAYASYPGASVDNYQQPPPTQNAETTEGYQPPPPY 224

```

The segment of gnlIPIDc1254905 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 177. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein.

- 5 Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

- Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 178 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Primary Dendritic Cells, lib 1 and to a lesser extent in Soares ovary tumor NbHOT; Primary Dendritic cells, frac 2; Breast Cancer Cell line, angiogenic; NCI_CGAP_GCB1; Soares_NFL_T_GBC_S1; Soares fetal liver spleen
- 15 1NFLS; Soares_total_fetus_Nb2HF8_9w; Soares_fetal_heart_NbHH19W; Spleen, Chronic lymphocytic leukemia; Soares_senescent_fibroblasts_NbHSF; Human Soleus; H Macrophage (GM-CSF treated), re-excision; Human Primary Breast Cancer Reexcision; NCI_CGAP_Co8; Soares infant brain 1NIB; Soares_fetal_Jung_NbHL19W; Healing groin wound - zero hr post-incision (control);
- 20 LNCAP prostate cell line; H. Lymph node breast Cancer; NCI_CGAP_Co9; Human Fetal Kidney; Human umbilical vein endothelial cells, IL-4 induced; Dendritic cells, pooled; Activated T-Cell (12hs)/Thiouridine labelledEco; NCI_CGAP_Kid5; Human Bone Marrow, treated; NCI_CGAP_Co8; Soares_fetal_heart_NbHH19W; NCI_CGAP_Co8; Human Primary Breast Cancer; Human Quadriceps; Human
- 25 Tonsils, Lib 2; pBMC stimulated w/ poly I/C; Stratagene placenta (#937225); Breast Cancer cell line, MDA 36; Human Pituitary, subt IX; NCI_CGAP_Co3;

- NCI_CGAP_Pr22; Human Thymus; Human Testes Tumor, re-excision; Soares breast 2NbHBst; CHME Cell Line, treated 5 hrs; Colon Tumor; Colon Carcinoma; Human Substantia Nigra; Human Placenta; Normal colon; Human Fetal Kidney, Reexcision; human tonsils; NCI_CGAP_Kid3; NCI_CGAP_Pr12; Hodgkin's Lymphoma II;
- 5 Human Resting Macrophage; H.Leukocytes, normalized cot 5B; Human Eosinophils; Human promyelocyte; Soares_NSF_F8_9W_OT_PA_P_S1; Soares_parathyroid_tumor_NbHPA; Soares_fetal_liver_spleen_1NFLS_S1; Stratagene lung carcinoma 937218; Human Amygdala Depression, re-excision; Human Pancreatic Langerhans; Bone Cancer; Human Kidney; Human epithelioid
- 10 sarcoma; Human (Caco-2) cell line, adenocarcinoma, colon, remake; Human Colon, subtraction; metastatic squamous cell lung carcinoma, poorly differentiated; Human Aortic Endothelium; Human colon carcinoma (HCC) cell line, remake; Hodgkin's Lymphoma I; Adipocytes, re-excision; NCI_CGAP_Ut3; NCI_CGAP_Co14; NCI_CGAP_Kid5; NCI_CGAP_Brn23; NCI_CGAP_Brn25; CD40 activated
- 15 monocyte dendritic cells; H. Epididymus, caput & corpus; H. cerebellum, Enzyme subtracted; Breast Lymph node cDNA library; Human Lung; LPS activated derived dendritic cells; Lung Carcinoma A549 TNFalpha activated; Cem cells cyclohexamide treated; Raji Cells, cyclohexamide treated; Soares_parathyroid_tumor_NbHPA; B Cell lymphoma; Human Epididymus; Hepatocellular Tumor; Healing groin wound,
- 20 7.5 hours post incision; Human endometrial stromal cells-treated with progesterone; Human Colon, re-excision; Synovial hypoxia; Healing groin wound, 6.5 hours post incision; Human Infant Brain; Mo7e Cell Line GM-CSF treated (1ng/ml); TF-1 Cell Line GM-CSF Treated; KMH2; NCI_CGAP_Co2; NCI_CGAP_Lu1; NCI_CGAP_Pr9; NCI_CGAP_Co12; NCI_CGAP_Kid6; NCI_CGAP_Pr10;
- 25 NCI_CGAP_Pr11; NCI_CGAP_Pr20; NCI_CGAP_Pr23; Monocyte activated, re-excision; L428; Human Uterine Cancer; Human Heart; Human Pancreas Tumor,

- Reexcision; Human Hippocampus; Olfactory epithelium, nasal cavity; Human Activated Monocytes; Ulcerative Colitis; Hemangiopericytoma; NCI_CGAP_Kid3; NCI_CGAP_Brn23; Ovarian Tumor 10-3-95; Hepatocellular Tumor, re-excision; Human Liver, normal; Stratagene liver (#937224); Pancreas Islet Cell Tumor; Resting
- 5 T-Cell Library, II; breast lymph node CDNA library; Colon Tumor II; Human fetal heart, Lambda ZAP Express; Soares melanocyte 2NbHM; Human Adult Pulmonary, re-excision; CD34 depleted Buffy Coat (Cord Blood), re-excision; Colon Normal III; Anergic T-cell; NCI_CGAP_Br2; NCI_CGAP_GC3; NCI_CGAP_HIN4; NCI_CGAP_Lu5; NCI_CGAP_CLL1; NCI_CGAP_Kid6; NCI_CGAP_Lu5;
- 10 NCI_CGAP_Brn23; T Cell helper I; Bone Marrow Cell Line (RS4,11); Activated T-cell(12h)/Thiouridine-re-excision; T cell helper II; Infant brain, LLNL array of Dr. M. Soares 1NIB; Human fetal heart, Lambda ZAP Express and Stratagene neuroepithelium (#937231).

- Preferred polypeptides of the present invention comprise immunogenic
- 15 epitopes shown in SEQ ID NO: 104 as residues: Asn-36 to Tyr-44. Polynucleotides encoding said polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:55 and may have been publicly available prior to conception of
- 20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1560 of SEQ ID NO:55, b
- 25 is an integer of 15 to 1574, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 46

- 5 The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gn||PID|d1033268 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "(AB007930)
- 10 KIAA0461 peroteine [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

```

>gnl|PID|d1033268 (AB007930) KIAA0461 peroteine [Homo sapiens]
>sp|O75049|O75049
15 KIAA0461 PEROTEIN (FRAGMENT).
   Length = 1355

   Plus Strand HSPs:

20 Score = 726 (255.6 bits), Expect = 5.1e-78, Sum P(2) = 5.1e-78
   Identities = 148/188 (78%), Positives = 148/188 (78%), Frame = +1

   Q: 67 KGMLVMDCHRTLHSEEVLAHLSASSTLPVAVPAGCSSKIQLDVCIKRTVKNFLHKKWKE 246
      KGMLVMDCHRTLHSEEVLAHLSASSTLPVAVPAGCSSKIQLDVCIKRTVKNFLHKKWKE
25 S: 1168 KGMLVMDCHRTLHSEEVLAHLSASSTLPVAVPAGCSSKIQLDVCIKRTVKNFLHKKWKE 1227

   Q: 247 QAREMADTACDSXXXXXXXXXXXXXXXXXXXXDCPELVQRSFLVASVLPDGNINSPTRN 426
      QAREMADTACDS DCPPELVQRSFLVASVLPDGNINSPTRN
25 S: 1228 QAREMADTACDSVLQLVLVWLGEVLGVIGDCPELVQRSFLVASVLPDGNINSPTRN 1287

30 Q: 427 ADMQEEELIASLEEQKLKSGXXXXXXXXXXXXXXXXXXXXLHQLFEGESETESFYGFEE 606
      ADMQEEELIASLEEQKLKSG LHQLFEGESETESFYGFEE
   S: 1288 ADMQEEELIASLEEQKLKSGHSESTPRPRSSPEETIEPELHQLFEGESETESFYGFEE 1347

35 Q: 607 ADLDLMEI 630
      ADLDLMEI
   S: 1348 ADLDLMEI 1355

   Score = 104 (36.6 bits), Expect = 5.1e-78, Sum P(2) = 5.1e-78
40 Identities = 18/22 (81%), Positives = 19/22 (86%), Frame = +2

   Q: 14 VELWSTRVWQKHTACQRXKACL 79
      +ELWSTRVWQKHTACQR K L

```


S: 1150 MELWSTRVWQKHTACQRSKGL 1171

The segments of gnlIPIDd1033268 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 179 and SEQ ID NO. 181. Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 180 and/or SEQ ID NO. 182 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Fetal Brain and to a lesser extent in Human 8 Week Whole Embryo; Human Amygdala; Nine Week Old Early Stage Human; Human Cerebellum; Soares_fetal_heart_NbHH19W; Soares infant brain 1NIB; Soares ovary tumor NbHOT; Soares_pregnant_uterus_NbHPU; Stratagene NT2 neuronal precursor 937230; H Female Bladder, Adult; Soares_fetal_lung_NbHL19W; Human Brain, Striatum; Human Hypothalamus, Schizophrenia; Rejected Kidney, lib 4; Stratagene lung (#937210); Soares_pregnant_uterus_NbHPU; H. Striatum Depression, sub; H. Atrophic Endometrium; Stratagene corneal stroma (#937222); Human Neutrophils, Activated, re-excision; NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Breast Cancer cell line, MDA 36; Soares_fetal_lung_NbHL19W; H. Kidney Cortex, subtracted; Human Colon, re-excision; Synovial hypoxia; Brain Frontal Cortex, re-excision; KMH2; L428; 12 Week Old Early Stage Human, II; Human Uterine Cancer; Stromal cell TF274; Bone Marrow Stromal Cell, untreated; Human Adrenal Gland

Tumor; Macrophage-oxLDL, re-excision; Human Ovarian Cancer Reexcision;
 Resting T-Cell Library,II; Human Placenta; Bone marrow; Endothelial-induced;
 Anergic T-cell; Soares_fetal_lung_NbHL19W; HUMAN B CELL LYMPHOMA;
 Human Bone Marrow, treated; Bone Marrow Cell Line (RS4,11); Human

- 5 Endometrial Tumor; Hodgkin's Lymphoma II and Soares fetal liver spleen 1NFLS.

Preferred polypeptides of the present invention comprise immunogenic
 epitopes shown in SEQ ID NO: 105 as residues: Glu-2 to Tyr-11. Polynucleotides
 encoding said polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly
 10 available and accessible through sequence databases. Some of these sequences are
 related to SEQ ID NO:56 and may have been publicly available prior to conception of
 the present invention. Preferably, such related polynucleotides are specifically
 excluded from the scope of the present invention. To list every related sequence
 would be cumbersome. Accordingly, preferably excluded from the present invention
 15 are one or more polynucleotides comprising a nucleotide sequence described by the
 general formula of a-b, where a is any integer between 1 to 2721 of SEQ ID NO:56, b
 is an integer of 15 to 2735, where both a and b correspond to the positions of
 nucleotide residues shown in SEQ ID NO:56, and where b is greater than or equal to a
 + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 47

It has been discovered that this gene is expressed primarily in the following
 tissues/cDNA libraries: Primary Dendritic Cells, lib 1 and to a lesser extent in
 Dendritic cells, pooled; Soares retina N2b4HR; Human Eosinophils;

- 25 Soares_pineal_gland_N3HPG; T-Cell PHA 24 hrs; Rejected Kidney, lib 4;

Soares_fetal_lung_NbHL19W; Human Neutrophil, Activated; Monocyte activated and Human Bone Marrow, treated.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 106 as residues: Ser-17 to Gly-22, Leu-34 to Ala-42.

- 5 Polynucleotides encoding said polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:57 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
10 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2717 of SEQ ID NO:57, b is an integer of 15 to 2731, where both a and b correspond to the positions of
15 nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 48

It has been discovered that this gene is expressed primarily in the following
20 tissues/cDNA libraries: Glioblastoma; Dendritic cells, pooled; Anergic T-cell; HUMAN B CELL LYMPHOMA; Spleen, Chronic lymphocytic leukemia; Activated T-cell(12h)/Thiouridine-re-excision; Primary Dendritic Cells, lib 1.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
25 related to SEQ ID NO:58 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

- excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1454 of SEQ ID NO:58, b
- 5 is an integer of 15 to 1468, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 49

- 10 The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil1872200 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "alternatively spliced
- 15 product using exon 13A [Homo sapiens]". A partial alignment demonstrating the observed homology is shown immediately below.

- ```

20 >gi|1872200 alternatively spliced product using exon 13A [Homo sapiens]
 >sp|P78525|P78525 MYB PROTO-ONCOGENE PROTEIN (C-MYB).
 Length = 666

 Minus Strand HSPs:

25 Score = 315 (110.9 bits), Expect = 1.1e-25, Sum P(2) = 1.1e-25
 Identities = 62/80 (77%), Positives = 64/80 (80%), Frame = -2

 Q: 1040 HDLGSPQLPPGPKRFSCLSISSSWDYRHAPPRPANFVFLVETGFLHVG*AGLKLPTSGD 861
 HD GS QLLPPGPKRFSCLS+ SWDYRH PPRPANF FLVETGFLHVG AGL+L TSGD
 S: 572 HDFGSLQLPPGPKRFSCLSLPRSWDYRHPPRPANFEFLVETGFLHVGAGLELLTSGD 631

30 Q: 860 PPAAWQSAGITGVXTAPGP 801
 PA ASQSA ITGV P
 S: 632 LPASASQSARITGVSHRARP 651

35 Score = 42 (14.8 bits), Expect = 1.1e-25, Sum P(2) = 1.1e-25
 Identities = 7/8 (87%), Positives = 7/8 (87%), Frame = -3

```

Q: 1063 AQTGVQNH 1040  
A TGVQNH  
S: 565 APTGVQNH 572

5                   The segments of gil1872200 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 183 and SEQ ID NO. 185 . Based on the structural similarity, these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in  
10 the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 184 and/or SEQ ID NO. 186 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed).

15               It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Cerebellum; NCI\_CGAP\_Ov2; Soares fetal liver spleen 1NFLS and to a lesser extent in Dermatofibrosarcoma Protuberance; Merkel Cells; Human Thymus Stromal Cells; Primary Dendritic cells, frac 2; Endothelial-induced; Stratagene neuroepithelium (#937231) and Primary Dendritic Cells, lib 1.

20               Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:59 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence  
25 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1600 of SEQ ID NO:59, b

is an integer of 15 to 1614, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.

Table 1

| Gene No. | cDNA Clone ID | ATCC Deposit No. Z and Date | Vector     | NT SEQ ID NO: X | NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of AA of Signal Pep | 5' NT of First AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|-----------------------------|------------|-----------------|---------|---------------------|---------------------|---------------------------|---------------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 1        | HE8FC45       | 203979<br>04/29/99          | Uni-ZAP XR | 11              | 1887    | 1                   | 1887                | 155                       | 155                             | 60              | 1                   | 34                 | 35                           | 47             |
| 2        | HETC16        | 203979<br>04/29/99          | Uni-ZAP XR | 12              | 2285    | 73                  | 2285                | 237                       | 237                             | 61              | 1                   | 27                 | 28                           | 40             |
| 3        | HETDD61       | 203979<br>04/29/99          | Uni-ZAP XR | 13              | 1805    | 2                   | 1805                | 148                       | 148                             | 62              | 1                   | 24                 | 25                           | 32             |
| 4        | HETDW58       | 203979<br>04/29/99          | Uni-ZAP XR | 14              | 1533    | 328                 | 1533                | 541                       | 541                             | 63              | 1                   | 18                 | 19                           | 22             |
| 5        | HETFO57       | 203979<br>04/29/99          | Uni-ZAP XR | 15              | 1758    | 64                  | 1755                | 195                       | 195                             | 64              | 1                   | 24                 | 25                           | 36             |
| 6        | HFCBL53       | 203979<br>04/29/99          | Uni-ZAP XR | 16              | 1901    | 237                 | 1838                | 307                       | 307                             | 65              | 1                   | 17                 | 18                           | 39             |
| 7        | HFIK29        | 203979<br>04/29/99          | pSport1    | 17              | 3145    | 1366                | 3145                | 1611                      | 1611                            | 66              | 1                   | 18                 | 19                           | 26             |

| Gene No. | cDNA Clone ID | ATCC Deposit No. Z and Date | Vector         | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of First AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|-----------------------------|----------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 8        | HJMAZ60       | 203979<br>04/29/99          | pCMV Sport 3.0 | 18              | 2968          | 1393                | 2915                | 1441                 | 1441                            | 67              | 1                   | 25                 | 26                           | 45             |
| 9        | HJMBB20       | 203979<br>04/29/99          | pCMV Sport 3.0 | 19              | 3953          | 2237                | 3953                | 2308                 | 2308                            | 68              | 1                   | 25                 | 26                           | 36             |
| 10       | HJPAZ35       | 203979<br>04/29/99          | Uni-ZAP XR     | 20              | 1963          | 665                 | 1940                | 832                  | 832                             | 69              | 1                   |                    |                              | 24             |
| 11       | HLHEE27       | 203979<br>04/29/99          | Uni-ZAP XR     | 21              | 3045          | 1107                | 2270                | 1260                 | 1260                            | 70              | 1                   | 23                 | 24                           | 61             |
| 12       | HLMA65        | 203979<br>04/29/99          | Lambda ZAP II  | 22              | 446           | 1                   | 446                 | 131                  | 131                             | 71              | 1                   |                    |                              | 10             |
| 13       | HAPNI33       | 203979<br>04/29/99          | Uni-ZAP XR     | 23              | 3397          | 1593                | 3376                | 1872                 | 1872                            | 72              | 1                   | 36                 | 37                           | 70             |
| 14       | HTXKB57       | 203979<br>04/29/99          | Uni-ZAP XR     | 24              | 2836          | 570                 | 2801                | 867                  | 867                             | 73              | 1                   | 16                 | 17                           | 61             |
| 15       | HBIBF26       | 203979<br>04/29/99          | Uni-ZAP XR     | 25              | 2101          | 591                 | 2089                | 655                  | 655                             | 74              | 1                   | 22                 | 23                           | 183            |



| Gene No. | cDNA Clone ID | ATCC Deposit No. Z and Date | Vector        | NT SEQ ID NO. X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of AA of Signal Pep | AA SEQ ID NO. Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|-----------------------------|---------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 16       | HCE3R46       | 203979<br>04/29/99          | Uni-ZAP XR    | 26              | 2046          | 58                  | 2025                | 376                  | 376                       | 75              | 1                   |                    |                              | 22             |
| 17       | HDLAL94       | 203979<br>04/29/99          | pCMVSPORT 2.0 | 27              | 3569          | 548                 | 3569                | 192                  | 192                       | 76              | 1                   | 15                 | 16                           | 179            |
| 18       | HLHDL69       | 203979<br>04/29/99          | Uni-ZAP XR    | 28              | 2556          | 364                 | 2556                | 433                  | 433                       | 77              | 1                   | 20                 | 21                           | 38             |
| 19       | HMVCG17       | 203979<br>04/29/99          | pSport1       | 29              | 1149          | 1                   | 1149                | 253                  | 253                       | 78              | 1                   | 22                 | 23                           | 65             |
| 20       | HMVCS92       | 203979<br>04/29/99          | pSport1       | 30              | 1867          | 1                   | 1730                | 323                  | 323                       | 79              | 1                   | 24                 | 25                           | 47             |
| 21       | HNALD94       | 203979<br>04/29/99          | pSport1       | 31              | 1272          | 1                   | 1249                | 88                   | 88                        | 80              | 1                   | 13                 | 14                           | 217            |
| 22       | HINEDQ02      | 203979<br>04/29/99          | Uni-ZAP XR    | 32              | 2235          | 131                 | 2235                | 280                  | 280                       | 81              | 1                   | 23                 | 24                           | 56             |
| 23       | HOECX21       | 203979<br>04/29/99          | Uni-ZAP XR    | 33              | 1963          | 56                  | 1892                | 75                   | 75                        | 82              | 1                   | 33                 | 34                           | 37             |

| Gene No. | cDNA Clone ID | ATCC Deposit No. Z and Date | Vector          | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of First AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|-----------------------------|-----------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 24       | HOSEJ94       | 203979<br>04/29/99          | Uni-ZAP XR      | 34              | 1767          | 622                 | 1750                | 848                  | 848                             | 83              | 1                   | 22                 | 23                           | 28             |
| 25       | HPJAB75       | 203979<br>04/29/99          | Uni-ZAP XR      | 35              | 1817          | 1                   | 1792                | 153                  | 153                             | 84              | 1                   |                    |                              | 28             |
| 26       | HPMEW01       | 203979<br>04/29/99          | Uni-ZAP XR      | 36              | 2488          | 1                   | 2215                | 95                   | 95                              | 85              | 1                   |                    |                              | 18             |
| 27       | HPMSH26       | 203979<br>04/29/99          | pBluescript     | 37              | 1758          | 47                  | 1697                | 322                  | 322                             | 86              | 1                   | 16                 | 17                           | 24             |
| 28       | HPQAX38       | 203979<br>04/29/99          | Lambda ZAP II   | 38              | 1158          | 41                  | 1158                |                      | 295                             | 87              | 1                   | 13                 | 14                           | 16             |
| 29       | HPWTF53       | 203979<br>04/29/99          | Uni-ZAP XR      | 39              | 2190          | 227                 | 2190                |                      | 484                             | 88              | 1                   | 18                 | 19                           | 97             |
| 30       | HRTAP63       | 203979<br>04/29/99          | pBluescript SK- | 40              | 2576          | 891                 | 2576                | 959                  | 959                             | 89              | 1                   | 28                 | 29                           | 42             |
| 31       | HSSJM47       | 203979<br>04/29/99          | Uni-ZAP XR      | 41              | 1152          | 1                   | 1000                |                      | 151                             | 90              | 1                   |                    |                              | 2              |

| Gene No. | cDNA Clone ID | ATCC Deposit No-Z and Date | Vector     | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|----------------------------|------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 32       | HASAU26       | 203979<br>04/29/99         | Uni-ZAP XR | 42              | 3853          | 1557                | 2090                | 1767                 | 1767                      | 91              | 1                   | 24                 | 25                           | 50             |
| 33       | HSIDA33       | 203979<br>04/29/99         | Uni-ZAP XR | 43              | 556           | 1                   | 535                 |                      | 196                       | 92              | 1                   |                    |                              | 4              |
| 34       | HSIFO61       | 203979<br>04/29/99         | Uni-ZAP XR | 44              | 2150          | 647                 | 2076                | 684                  | 684                       | 93              | 1                   | 33                 | 34                           | 36             |
| 35       | HPWTF23       | 203979<br>04/29/99         | Uni-ZAP XR | 45              | 2008          | 94                  | 1994                | 283                  | 283                       | 94              | 1                   | 30                 | 31                           | 130            |
| 36       | HSLHG78       | 203979<br>04/29/99         | Uni-ZAP XR | 46              | 1474          | 452                 | 1474                | 647                  | 647                       | 95              | 1                   | 20                 | 21                           | 70             |
| 37       | HSSJW30       | 203979<br>04/29/99         | Uni-ZAP XR | 47              | 1893          | 581                 | 1880                |                      | 713                       | 96              | 1                   | 15                 | 16                           | 24             |
| 38       | HTEDT87       | 203979<br>04/29/99         | Uni-ZAP XR | 48              | 2418          | 726                 | 2418                | 949                  | 949                       | 97              | 1                   |                    |                              | 17             |
| 39       | HTLFI73       | 203979<br>04/29/99         | Uni-ZAP XR | 49              | 1159          | 1                   | 1159                | 340                  | 340                       | 98              | 1                   |                    |                              | 23             |

| Gene No. | cDNA Clone ID | ATCC Deposit No:Z and Date | Vector          | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of First AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Secreted Portion | Last AA of ORF |
|----------|---------------|----------------------------|-----------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------------|-----------------|---------------------|-----------------------------|----------------|
| 40       | HTNB15        | 203979<br>04/29/99         | pBluescript SK- | 50              | 2792          | 1511                | 2763                | 1737                 | 1737                            | 99              | 1                   | 22                          | 51             |
| 41       | HTOET03       | 203979<br>04/29/99         | Uni-ZAP XR      | 51              | 1921          | 434                 | 1897                | 518                  | 518                             | 100             | 1                   |                             | 24             |
| 42       | HTXJW06       | 203979<br>04/29/99         | Uni-ZAP XR      | 52              | 1494          | 511                 | 1494                | 635                  | 635                             | 101             | 1                   | 20                          | 129            |
| 43       | HUKAI28       | 203979<br>04/29/99         | Lambda ZAP II   | 53              | 1700          | 1                   | 1700                | 61                   | 61                              | 102             | 1                   | 16                          | 32             |
| 44       | HUKFC71       | 203979<br>04/29/99         | Lambda ZAP II   | 54              | 1177          | 1                   | 973                 | 261                  | 261                             | 103             | 1                   |                             | 9              |
| 45       | HUKFV37       | 203979<br>04/29/99         | Lambda ZAP II   | 55              | 1574          | 20                  | 1531                | 49                   | 49                              | 104             | 1                   | 45                          | 224            |
| 46       | HUKFY09       | 203979<br>04/29/99         | Lambda ZAP II   | 56              | 2735          | 1                   | 957                 | 237                  | 237                             | 105             | 1                   |                             | 13             |
| 47       | HWBBU75       | 203979<br>04/29/99         | pCMV Sport 3.0  | 57              | 2731          | 623                 | 2731                | 783                  | 783                             | 106             | 1                   | 22                          | 51             |

| Gene No. | cDNA Clone ID | ATCC Deposit No. Z and Date | Vector         | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|-----------------------------|----------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 48       | HWBEF34       | 203979<br>04/29/99          | pCMV Sport 3.0 | 58              | 1468          | 1                   | 1468                | 134                  | 134                 | 107             | 1                   | 31                 | 32                           | 44             |
| 49       | HEMEY47       | 203979<br>04/29/99          | Uni-ZAP XR     | 59              | 1614          | 204                 | 1614                | 440                  | 440                 | 108             | 1                   |                    |                              | 10             |

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is

identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted

translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods.

- 5 The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

- 10 The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate  
15 sources of genomic material.

- Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a  
20 deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

- 25 The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly



produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

#### **Signal Sequences**

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, *Virus Res.* 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra.*) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

**Polynucleotide and Polypeptide Variants**

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA  
5 sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential  
10 properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide  
15 coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence contained in a deposited cDNA clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of  
20 these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or  
25 alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence

shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95%  
5 "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95%  
10 identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified  
15 as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best  
20 overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by  
25 converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to

calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

5            If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent  
10   identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using  
15   the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

20            For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number  
25   of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly

matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table I (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject

sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.



For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10  
5 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100  
10 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not  
15 matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter  
20 the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host  
25 (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500

nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used.

- 5 (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

- As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic
- 10 residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

- Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues,
- 20 where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino
- 25 acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory

sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

## **Polynucleotide and Polypeptide Fragments**

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the

complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the

mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a



polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

- 5           The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, 15 precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary 20 antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the 25 invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel

chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

5 In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope  
10 of the invention.

### Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID  
15 NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined *supra*. The present invention further encompasses  
20 polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower  
25 stringency hybridization conditions defined *supra*.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide

5 encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a  
10 portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

15 Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10,  
20 at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes  
25 include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies,

that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a

carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or

5 intradermal injection of emulsions containing about 100  $\mu$ g of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid

10 surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the

15 polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins

20 may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4- polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the

25 immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO

- 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 5 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, 10 Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup>-
- 15 nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

- Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to
- 20 modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in

its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

### Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM,

IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).



Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in  
5 contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in  
10 terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described  
15 herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%,  
20 less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific  
25 antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides

which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described

supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

5           The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as  
10       well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the  
15       receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al.,  
25       Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998);

Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques,

including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone,

including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the

5 Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, 10 for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

15 Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the 20 hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such 25 as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments).

F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

- For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.
- As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies,

including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; 5 Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule 15 in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which 20 are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. 25 Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve,



- antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g.,
- 5 Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP
- 10 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

- Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods
- 15 known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

- 20 Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the
- 25 human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes.

The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a

selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, J. *Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

#### Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the

nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence  
5 encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is  
10 known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable  
15 to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

20 Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor,  
25 NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John

Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

- In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.* 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

- In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci.* 81:851-855 (1984); Neuberger et al., *Nature* 312:604-608 (1984); Takeda et al., *Nature* 314:452-454 (1985)) by splicing genes

from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

#### Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the

antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be  
5 used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or  
10 light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or  
15 light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain  
20 thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the  
25 antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently

purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the



generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region

E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the

5 ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate

10 transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g.,

15 cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which

20 possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell

25 line such as, for example, CRL7030 and Hs578Bst.

- For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.
- 15 A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp<sup>r</sup>t- or apt<sup>r</sup>- cells, respectively.
- 20 Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-
- 25 418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science

260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, 1993, *TIB TECH* 11(5):155-215; and hygromycin, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), *Current Protocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin et al., *J. Mol. Biol.* 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.* 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci.*

USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS

89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins

consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include



paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and

5 puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-

10 dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological

15 response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth

20 factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g.,

25 angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),

granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

### Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,

complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

- Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasyolol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

- Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-

fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., <sup>32</sup>P or <sup>125</sup>I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al,

eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., <sup>3</sup>H or <sup>125</sup>I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., <sup>3</sup>H or <sup>125</sup>I) in the presence of increasing amounts of an unlabeled second antibody.

#### 15      Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment

and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC).

Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

#### Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217



(1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY  
5 (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters  
10 operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the  
15 antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

20 Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

25 In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be

accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct  
5 injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see,  
10 e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for  
15 cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al.,  
20 Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral  
25 genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which

facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy.

- 10 Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

- Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

10 In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and  
15 maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of  
20 gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are  
25 preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the

therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)),

construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein

and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g.,



Joliet et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable

pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant

expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect,

diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody

assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

5           One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which  
10           specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that  
15           detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

20           It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially  
25           accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of

Radiolabeled Antibodies and Their Fragments.” (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode  
5 of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

10 In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods  
15 known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging  
20 (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive  
25 scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In

yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

### Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by  
5 binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of  
10 the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

15 In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of  
20 bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

25 The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip



sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group.

- 5 Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting  
10 surface-bound anti-antigen antibody.

### **Fusion Proteins**

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a  
15 second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

20 Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics  
25 of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the

polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling

5 of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in

10 chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).)

15 Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion

20 proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified,

25 would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for

example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

- 5           Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available.
- 10          As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)
- 15          Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

#### **Vectors, Host Cells, and Protein Production**

- 20          The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

- 25          The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate,

such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate  
5 promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding  
10 portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin  
15 resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178));  
20 insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-  
9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A,  
25 pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech,

Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, 5 pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated 10 transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

15 A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most 20 preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical 25 synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant,

insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using  $O_2$ . This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for  $O_2$ . Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast* 5:167-77 (1989); Tschopp, J.F., et al., *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1*

regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide

sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteine acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino



acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, *e.g.*, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random

positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

- One may specifically desire proteins chemically modified at the N-terminus.
- Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein
- 5 (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules.
- 10 Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl
- 15 group containing polymer is achieved.

- The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific
- 20 embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

- Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides
- 25 corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and

fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional  
5  
10  
15  
embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the  
15  
invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as,  
20  
for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the  
25  
invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the

invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper

polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different

5 proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant

10 fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are

15 those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

20 In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations

proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-

25 Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic

polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described  
5 herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

10

#### Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

15

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

20

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids  
25 containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.



Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and

one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined.

- 5 First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.
- 10

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using

- 15 polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

20

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe

25

containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand  
5 containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present  
10 invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the  
15 polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard  
20 being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

25 By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the

polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose

derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)).

- 5 Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., supra) It is likely that mutated or altered expression of specific genes is  
10 involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., supra)

- For example, c-myc expression is highly amplified in the non-lymphocytic leukemia  
15 cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb  
20 proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of  
25 hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

- In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.
- Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more  
5 restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

- 10 The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a  
15 unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

- Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological  
20 samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQA class II HLA gene, are used in forensic biology to identify individuals. (Erich, H., PCR  
25 Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an



identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### **Uses of the Polypeptides**

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene

expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein.

In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging:

The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can  
5 activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also  
10 be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

#### 15 Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present  
20 invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

25 Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a

polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Beldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J.

- 5 Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are
- 10 arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver,

15 and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry

20 into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and

25 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene;

- 5 pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

- Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters
- 10 include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters,
- 15 such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

- Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the
- 20 polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

- The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver,
- 25 spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye,

gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

5           The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

          In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant  
10          invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA ,  
15          84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

20          Cationic liposomes are readily available. For example, N[1-2,3-di(octyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially  
25          available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).



Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to

produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred.

- 5 The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology*, 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated.
- 10 SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and
- 15 then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta*, 394:483 (1975); Wilson et al., *Cell*, 17:77 (1979)); ether injection (Deamer et al., *Biochim. Biophys. Acta*, 443:629 (1976); Ostro et al., *Biochem. Biophys. Res. Commun.*, 76:836 (1977); Fraley et al., *Proc. Natl. Acad. Sci. USA*, 76:3348 (1979)); detergent dialysis (Enoch et al., *Proc. Natl. Acad. Sci. USA*, 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., *J. Biol. Chem.*, 255:10431 (1980);
- 20 Szoka et al., *Proc. Natl. Acad. Sci. USA*, 75:145 (1978); Schaefer-Ridder et al., *Science*, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports  
5 on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622,  
10 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding  
15 polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

20 The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, 1:5-14 (1990), which is incorporated herein by  
25 reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation,

the use of liposomes, and  $\text{CaPO}_4$  precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which  
15 include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can  
10 be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for  
15 many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science, 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt  
20 to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA, 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993); Rosenfeld et al., Cell, 68:143-155 (1992); Engelhardt et al., Human Genet. Ther.,  
25 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature, 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein

incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express E1a and E1b, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found

in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous

polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR.

Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth

factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein.

5 Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

10 Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available  
15 depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the  
20 rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting  
25 the composition centimeters and preferably, millimeters within arteries.



Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue

5    inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a

10    particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad.

15    Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a

20    polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise

25    condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of

polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and  
5 birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

### **Biological Activities**

The polynucleotides or polypeptides, or agonists or antagonists of the present  
10 invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

15

### **Immune Activity**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by activating or inhibiting the proliferation,  
20 differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer or some autoimmune diseases,  
25 disorders, and/or conditions, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present

invention can be used as a marker or detector of a particular immune system disease or disorder.

- A polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. A polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

- Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies), blood platelet diseases, disorders, and/or conditions (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used

to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating, preventing, and/or diagnosing autoimmune diseases, disorders, and/or conditions. Many autoimmune diseases, disorders, and/or conditions result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune diseases, disorders, and/or conditions.

Examples of autoimmune diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the

present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide or agonists or antagonist may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

#### **Hyperproliferative Disorders**

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders,

and/or conditions, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other  
5 cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may  
10 be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

Examples of hyperproliferative diseases, disorders, and/or conditions that can  
15 be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral),  
20 lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative diseases, disorders, and/or conditions include, but are not limited to:  
25 hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's

Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating or preventing cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating or preventing cell-proliferative diseases, disorders, and/or conditions in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the

beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing  
5 expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the  
10 normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any  
15 other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA  
20 delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in  
25 the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes



needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell  
5 proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or  
10 disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of  
15 the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of  
20 the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a  
25 mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for

producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

5 A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings  
10 provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing  
15 cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or  
20 hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and  
25 therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies,

- fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

- Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

- Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the

activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues.

10 Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be

15 achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells

20 expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are

25 useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present

invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

## 5            **Cardiovascular Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular  
10 abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome,  
15 levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular diseases, disorders, and/or conditions also include heart  
20 disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular  
25 septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous),

pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

- 5 Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include
- 10 paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

- Heart valve disease include aortic valve insufficiency, aortic valve stenosis,
- 15 hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

- Myocardial diseases include alcoholic cardiomyopathy, congestive
- 20 cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

- Myocardial ischemias include coronary disease, such as angina pectoris,
- 25 coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia

(including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and  
15 thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion  
10 injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention,  
15 are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle  
20 accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a *Therapeutic*, described in more detail below. Methods of delivering polynucleotides of the  
25 invention are described in more detail herein.



### Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which

5 neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes

10 pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995);

15 Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have

20 accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or

25 antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the

- invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating, preventing, and/or diagnosing an
- 5 angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat or prevent a cancer or tumor.
- 10 Cancers which may be treated, prevented, and/or diagnosed with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma;
- 15 Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.
- 20 Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate
- 25 mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or conditions include, but are not limited to: benign tumors, for example hemangiomas, 5 acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed 10 wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound 15 granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

20 Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably 25 initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted

above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

5           Moreover, Ocular diseases, disorders, and/or conditions associated with neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity  
10       macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

          Thus, within one aspect of the present invention methods are provided for  
15       treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain  
20       pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of diseases, disorders, and/or conditions can result in corneal neovascularization,  
25       including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and

Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

- Within particularly preferred embodiments of the invention, may be prepared
- 5 for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within
- 10 preferred embodiments, the anti-angiogenic composition is prepared with a mucoadhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic
- 15 response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

- Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance.
- 20 The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbal corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly
- 25 after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbal cornea interspersed

between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating or preventing neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a

patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

- 5           Additionally, diseases, disorders, and/or conditions which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, 10    pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

- Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for 15    example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, 20    hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth 25    control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence

such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in



order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

- 5        Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate  
10       including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

- Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten  
15       oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum  
20       (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

- A wide variety of other anti-angiogenic factors may also be utilized within the  
25       context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells),

- (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L- 3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin
- 10 (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-
- 15 316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxyaminolimidazole; and metalloproteinase inhibitors such as BB94.

### **Diseases at the Cellular Level**

- Diseases associated with increased cell survival or the inhibition of apoptosis
- 20 that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular
- 25 cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast

cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

- 10 Additional diseases or conditions associated with increased cell survival that could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia
- 15 (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited
- 20 to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal
- 25 cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary

carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, 5 craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, 10 and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and 15 immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic 20 shock, cachexia and anorexia.

#### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists 25 or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing,

and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye  
5 tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and  
10 antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and  
15 to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft,  
20 epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the  
25 appearance of aged skin.

It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or

agonists or antagonists of the invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the



invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

5           The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins  
10 known in the art).

          In addition, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the  
15 invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

20

### Neurological Diseases

          Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not  
25 limited to, nervous system injuries, and diseases, disorders, and/or conditions which

- result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of
- 5 either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the
  - 10 nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated
  - 15 with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6)
  - 20 lesions associated with nutritional diseases, disorders, and/or conditions, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar
  - 25 degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus

- erythematous, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency
- 5 virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

- In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the
- 10 invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or
- 15 antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral infarction. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists
- 20 or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

- The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of
- 25 limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture;

- (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

- In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include, but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-

Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary  
Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

### **Infectious Disease**

5           A polypeptide or polynucleotide and/or agonist or antagonist of the present  
invention can be used to treat, prevent, and/or diagnose infectious agents. For  
example, by increasing the immune response, particularly increasing the proliferation  
and differentiation of B and/or T cells, infectious diseases may be treated, prevented,  
and/or diagnosed. The immune response may be increased by either enhancing an  
10       existing immune response, or by initiating a new immune response. Alternatively,  
polypeptide or polynucleotide and/or agonist or antagonist of the present invention  
may also directly inhibit the infectious agent, without necessarily eliciting an immune  
response.

          Viruses are one example of an infectious agent that can cause disease or  
15       symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or  
polypeptide and/or agonist or antagonist of the present invention. Examples of  
viruses, include, but are not limited to Examples of viruses, include, but are not  
limited to the following DNA and RNA viruses and viral families: Arbovirus,  
Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae,  
20       Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae  
(Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes  
Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae),  
Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papilloma  
virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or  
25       Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus),  
and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a

- variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis,
- 5 opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or
- 10 diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available
- 15 hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

- Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide
- 20 and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillus, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia
- 25 (e.g., Borrelia burgdorferi), Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic

- E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter,
- 5 Gonorrhea, Meningococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Hemophilus (e.g., Hemophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiocoecal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal
  - 10 families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease,
  - 15 Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections.
  - 20 Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diphtheria, botulism, and/or meningitis type B.
  - 25 Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or

antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g.,

- 5 Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or
- 10 polypeptides, or agonists or antagonists of the invention, can be used totreat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

- Preferably, treatment or prevention using a polypeptide or polynucleotide
- 15 and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a
- 20 vaccine to raise an immune response against infectious disease.

### **Regeneration**

- A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the
- 25 regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects,



trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs  
5 (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the  
10 present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel  
15 syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to  
20 proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral  
25 neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease,

Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

## 5 **Chemotaxis**

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as  
10 inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection,  
15 hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can  
20 be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used to treat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the  
25 present invention could be used as an inhibitor of chemotaxis.

**Binding Activity**

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or

more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF),  
5 insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-  
10 derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a  
15 decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H]  
20 thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is  
25 measured by liquid scintillation chromatography which measures the incorporation of

3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled  
5 polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is  
10 measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat, prevent, and/or  
15 diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the  
20 invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has  
25 been altered.

Also, one could identify molecules bind a polypeptide of the invention

experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one of the polypeptide sequences of the invention.

#### 15 Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for



delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

- In another embodiment, the invention provides a method for the specific
- 5 destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

- By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of
- 10 toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic
- 15 effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the
- 20 methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

## 25 Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or

the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the  
5 activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in  
10 solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present  
15 invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex  
20 between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

25 Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present

invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein.

Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The

5 peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

10 This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the  
15 invention.

#### Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the  
20 complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).

25 Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for

example, in Okano, *Neurochem.*, 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research*, 6:3073 (1979); Cooney et al., *Science*, 241:456 (1988); and Dervan et al., *Science*, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or

a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

- 5 Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible
- 10 or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445 (1981), the regulatory sequences of the
- 15 metallothionein gene (Brinster et al., *Nature*, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a

20 sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the

25 hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be).

One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should  
5 work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non-translated, non-coding regions of a polynucleotide sequence of the invention  
10 could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the  
15 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric  
20 mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell  
25 membrane (see, *e.g.*, Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.*, 84:648-652 (1987); PCT Publication

- NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., *BioTechniques*, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, *Pharm. Res.*, 5:539-549 (1988)). To this end,
- 5 the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

- The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil,
- 10 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine,
- 15 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil,
- 20 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

- The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose,
- 25 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

- In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)).
- 10 The oligonucleotide is a 2-0-methylribose nucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

- Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are
- 15 commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

- 20 While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

- Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published
- 25 October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs



corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases:

- 5    5' -UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5'
- 10    end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

- As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should
- 15    be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example,
- 20    pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

- Antagonist/agonist compounds may be employed to inhibit the cell growth
- 25    and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or

prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

10 The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

15 invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention

## 20 Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

25

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

5       The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may  
10       be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

15       The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

20       The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

25       The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or  
5 polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by  
10 influencing biorhythms, circadian rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present  
15 invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

### **Other Preferred Embodiments**

20 Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous  
25 nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of

positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous  
5 nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of  
10 contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

15 A further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide  
sequence which is at least 95% identical to a sequence of at least about 500  
20 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a  
nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ  
ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of  
the First Amino Acid of the Signal Peptide and ending with the nucleotide at about  
25 the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X  
in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under  
5 stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which  
10 comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide  
15 sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of  
20 at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

25 A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at

least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete  
5 nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X  
10 wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and  
15 determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence  
20 selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

25 A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic

acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least



95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise  
5 a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1  
10 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the  
15 amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the  
20 Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid  
25 sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1

and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at

least 10 contiguous amino acids in a sequence selected from the group consisting of:  
an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in  
Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA  
clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit  
5 with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences  
is performed by comparing the amino acid sequence determined from a polypeptide  
molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a  
10 biological sample which method comprises a step of detecting polypeptide molecules  
in said sample, if any, comprising an amino acid sequence that is at least 90%  
identical to a sequence of at least 10 contiguous amino acids in a sequence selected  
from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is  
any integer as defined in Table 1; and a complete amino acid sequence of a secreted  
15 protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in  
Table 1 and contained in the deposit with the ATCC Deposit Number shown for said  
cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell  
type of a biological sample, which method comprises a step of detecting polypeptide  
20 molecules comprising an amino acid sequence in a panel of at least two amino acid  
sequences, wherein at least one sequence in said panel is at least 90% identical to a  
sequence of at least 10 contiguous amino acids in a sequence selected from the above  
group.

Also preferred is a method for diagnosing in a subject a pathological condition  
25 associated with abnormal structure or expression of a gene encoding a secreted  
protein identified in Table 1, which method comprises a step of detecting in a

biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone

identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 2, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 2 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 2. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 2. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 2

| Gene No. | cDNA Clone ID | NT SEQ ID NO: X | Contig ID | Public Accession Numbers                                                                                                                                                                            |
|----------|---------------|-----------------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1        | HE8FC45       | 11              | 845672    | AA601208, AA804480, D44965, D44966, AA643257, AA076762, AA077478, AA078234, AA078336, AA078438, AA634301                                                                                            |
| 2        | HETCI16       | 12              | 844543    | T95778, T95779, H08007, H18947, H19390, R95854, R95853, N26576, N31078, N31550, N33885, N39638, N50035, N54692, N59356, N63480, N94778, W61061, W72796, W73600, W73639, W76118, AA010855, AA126946, |

|   |         |    |        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|---|---------|----|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|   |         |    |        | AA127155, AA156032, AA156202, AA863334, AA932906, AA987664, N76457, AA405125, AA405124, AA909484, A1073876, A1092369, Z39408                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 3 | HETDD61 | 13 | 800668 | N52797, N57619, N58971, W87476, W87571                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 4 | HETDW58 | 14 | 790557 | T93602, R09389, R01681, R50906, R60615, R60626, R69711, H29463, N53918, N75627, W44679, W93921, AA011658, AA045036, AA099321, AA143151, AA151528, AA150234, AA165256, AA424453                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 5 | HETFO57 | 15 | 844190 | N34796, N36401, N44277, N44518, N49951, N52732, N54323, W88968, W88526, W88547, W88607, AA113442, AA195526, AA226957, AA418598, AA418662, AA281630, AA281691, AA481407, AA481640, AA688082, AA743141, AA249323, AA722313, AA897363, A1056786, AA693840                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 6 | HFCBL53 | 16 | 845800 | R35376, R40315, R49183, R49678, R52614, R53269, R49183, R40315, R49678, R58993, R59051, H97961, N20667, N29044, AA228147, AA460466, AA829782, AA987933, AA885400, T03340, T03823, A1003191, T15737, T15845, T15879, T24788, Z38574, Z39335, Z42034, Z43266, F02507, F06226, AA772841                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 7 | HFIUK29 | 17 | 843587 | H68601, N29576, N42901, AA011654, AA032214, AA033669, AA035237, AA417266, AA514412, AA580190, AA834342, AA876544, AA417303, AA442413, AA456668, Z19172, AA928184, A1014558, A1093245, Z28430, Z38653, Z42935                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 8 | HJMAZ60 | 18 | 844796 | T48679, T48678, T78083, T78250, R25647, R25658, R25740, R27494, R27510, R28609, R32527, R35445, R75982, R76813, R79989, R80092, N26317, N32937, N35109, N39760, N42608, N43853, N62754, N79702, N95815, W24942, W93042, AA054425, AA074378, AA075972, AA113164, AA112425, AA128486, AA129797, AA191421, AA191552, AA235504, AA419262, AA419313, AA422127, AA506884, AA548792, AA552501, AA588403, AA568586, AA632390, AA573741, AA578870, AA565758, AA565783, AA662704, AA665375, AA737814, AA738377, AA744892, AA742230, AA809882, AA864231, AA876470, AA877488, AA932846, AA935484, AA937676, AA406562, AA411740, AA496304, AA504894, AA504946, AA868134, AA923223, A1091816, Z41515, AA952966, AA694309 |
| 9 | HJMBB20 | 19 | 844890 | T93306, T93982, R70875, R70874, R73649, R73730, N26796, N39485, AA004609, AA042801, AA134550, AA134551, AA182001, AA188081, AA210803, AA223397, AA223681, AA243523, AA465301, AA521126, AA553516, AA713955, AA741569, AA765139, AA766506, AA766608, AA767032, AA810250, AA810931, AA831595, W27345, AA641746, AA095711, AA248504,                                                                                                                                                                                                                                                                                                                                                                          |



|    |         |    |        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|----|---------|----|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |         |    |        | AA248975, D11999, AA598568, AA723133, D30931                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 10 | HJPAZ35 | 20 | 845379 | T49412, T49413, T50367, T51058, T54039, T57891, T57973, T60251, T61632, T62526, T62599, T63503, T63578, R20456, R35678, R35789, R44159, R44159, H17665, R86039, R97654, H57892, H63004, H68447, H78242, H78441, H94371, N21476, N22925, N23247, N23253, N25257, N31313, N33893, N35326, N44698, N45680, N48964, N52996, N79794, W01869, W04604, W06914, W07312, W32360, W43001, W45202, W52086, W79307, W79388, W93107, AA034229, AA057549, AA057688, AA065194, AA074555, AA074538, AA075218, AA075454, AA076573, AA076572, AA079405, AA079456, AA080910, AA082069, AA084344, AA085732, AA102668, AA101287, AA101375, AA101374, AA113221, AA112553, AA112627, AA113806, AA1130637, AA130740, AA173386, AA176464, AA176733, AA187481, AA187089, AA193530, AA424292, AA424488, AA225665, AA278364, AA281327, AA470408, AA470587, AA482674, AA493888, AA514294, AA523947, AA528797, AA530880, AA530893, AA531085, AA531481, AA531541, AA535285, AA548069, AA554315, AA558086, AA581419, AA583891, AA595287, AA600984, AA603713, AA605163, AA613369, AA612587, AA613931, AA627744, AA632739, AA573784, AA578084, AA568160, AA732046, AA747054, AA749070, AA765762, AA767269, AA768878, AA807539, AA825227, AA827578, AA832264, AA856555, AA862804, AA865752, AA906679, AA932220, AA970427, AA970773, AA973686, AA973949, AA996193, AA947999, W04240, W27772, AA092175, AA205439, AA651957, AA651984, AA654240, AA406193, AA410370, AA410369, AA436389, AA456775, AA481331, AA486584, AA486684, AA487547, AA491489, AA598684, AA598819, AA634643, AA644703, AA663212, AA670358, AA679993, AA456729, AA677303, AA678016, AA678879, AA705370, AA777757, AA781151, AA844663, AA845867, AA773557, A1003066, A1061259, A1075148, A1079490, T16086, T20215, T20214, AA693336 |
| 11 | HLHEE27 | 21 | 815512 | T52540, T64452, T65723, T89492, T89665, R00060, R06718, R54604, R55309, R55409, R73528, H25831, H25947, H25982, H26196, R94401, R96898, H65054, H65053, H83705, H98550, N20550, N29262, N34576, N71345, N71395, W01945, W02717, W25736, W67696, W68155, W69133, W69188, W74570, W74754, W90133, AA009844, AA044089, AA044218, AA058964, AA076132, AA075969                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 13 | HAPNJ33 | 23 | 835554 | T59657, T59790, T68171, R19341, R44614, R44614, H57782, H98612, N34568, N45212, N93001, W01101, W15371, W21212, W39402,                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

|    |         |    |        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|----|---------|----|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |         |    |        | W46789, W60640, AA062678, AA062679, AA074063, AA131690, AA134786, AA152198, AA150353, AA190706, AA191526, AA195382, AA531298, AA532687, AA533292, AA614001, AA614349, AA627431, AA639435, AA573295, AA574092, AA575905, AA662465, AA748473, AA766890, AA808328, AA814337, AA824621, AA856680, AA865162, AA887935, AA902159, AA915900, AA922613, AA946973, AA995156, AA995507, AI015364, F18935, W26679, AA642554, AA643647                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 14 | HTXKB57 | 24 | 844329 | R18404, R33853, R35962, R50693, R73315, H58842, N28447, N33030, N95714, W24871, W86697, AA007548, AA019726, AA021562, AA069819, AA160461, AA553530, H58790, H67263, H95601, AA847535, N42091, N68903, AA007549, C03610, AA021613, AA069909, C20848, AA401285, AA404278, C75020, AA779826, AA844486, AI040417, AI122741, Z43505                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 15 | HBIBF26 | 25 | 845743 | T56902, T56903, T77366, R48323, R48432, R69764, H06603, H06633, H08477, H08759, R87305, R87304, H52166, H89737, W56183, AA001019, AA001020, AA018345, AA018346, AA026966, AA482598, AA593837, AA612852, AA614565, AA576510, AA768838, AA814595, AA876261, AI000311, W22979, W56260, AA450329, AA450330, AA451942, AA971138, AI001995                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 16 | HCE3R46 | 26 | 844450 | T51838, T51884, T92411, T65552, T65619, T75302, T80328, T81433, T81434, T96979, T97091, R00998, R07112, R13827, R13922, R27577, R27676, R35406, R37686, R38011, R38672, R39034, R49203, R51542, R53033, R49203, R61540, R61594, H00151, H01276, H05079, H07042, H07041, H14588, H14693, H16360, H37896, H38255, H99405, N21123, N31138, N34542, N75155, N80252, W07378, W42462, W45201, W47578, W47599, W56427, W74265, W79879, W90765, W90698, AA001746, AA001816, AA004544, AA021240, AA021298, AA069253, AA069252, AA075721, AA075772, AA081577, AA084448, AA086275, AA099494, AA102004, AA112823, AA113108, AA128022, AA135400, AA137149, AA150268, AA150370, AA165393, AA165473, AA181003, AA182857, AA187989, AA187990, AA259097, AA224849, AA224936, AA259252, AA259253, AA468580, AA528583, AA554940, AA593620, AA622124, AA627792, AA570061, AA565937, AA658029, AA714075, AA729824, AA731839, AA737425, AA825318, AA837815, AA862234, AA862451, AA865629, AA887005, AA887194, AA927428, AA946668, AA946797, AA970342, AA983168, AI085303, N55999, N56531, W22064, W22147, W22225, W22333, W22643, N88963, C05903, AA090863, AA091082, AA093823, AA206334, |

|    |         |    |        |                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----|---------|----|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |         |    |        | AA207160, AA292871, AA401686, AA406410, AA410351, D12239, AA488382, AA488514, AA585103, AA653606, AA669989, AA706657, AA709303, AA776685, AA812651, Z19302, AA843667, T03456, AA984576, A1076144, A1076537, A1084406, T10532, Z28606, Z28736, Z28871, T24105, T16320, D31276, F11118, F00225, F13118, F13539, F10722                                                                                                                                 |
| 17 | HDLAL94 | 27 | 843583 | N48364, AA430064, AA922226, AA939344, A1079331                                                                                                                                                                                                                                                                                                                                                                                                       |
| 18 | HLHDL69 | 28 | 843836 | T60023, R00439, R51893, R54281, H65908, H73031, H88051, H88262, H88262, W40166, W45294, W53019, W65479, W65430, AA009923, AA031619, AA031728, AA046897, AA046962, AA076314, AA076486, AA111854, AA234173, AA287817, AA534598, AA761785, AA810858, AA827338, AA830471, AA887506, AA938723, AA972358, AA973851, AA453971, AA478573, AA478708, C75311, AA599138, AA644330, AA708726, AA721966, AA732750, AA772486, AA913468, A1033131, A1088217, T10188 |
| 19 | HMVCG17 | 29 | 846161 | R66124, AA256647, AA281722, AA507043, AA515030, AA741576, AA810762, AA812147, AA862554, AA863055, AA256664, AA481439, A1016577, F05632, R00171                                                                                                                                                                                                                                                                                                       |
| 20 | HMVCS92 | 30 | 846141 | R31321, R31580, H94502, H94586, W56135, AA683125, AA747095, AA807676, AA836406, AA402145, AA411797, AA677446, AA707609, AA702187                                                                                                                                                                                                                                                                                                                     |
| 21 | HNALD94 | 31 | 844722 | H60436, H84776, AA570089, AA894833, AA922599, AA926958, AA043129, AA218629, AA232816, AA233870, AA411348, AA447193, AA454745, AA706438, T15691                                                                                                                                                                                                                                                                                                       |
| 22 | HNEDQ02 | 32 | 846061 | W31293, AA178924, AA179049, AA417583, AA417691, AA514351, AA551669, AA573433, AA857884, AA858111, AA446763, AA446879, AA447224, AA432078, AA758017, AA845692, AA921924                                                                                                                                                                                                                                                                               |
| 23 | HOECX21 | 33 | 842866 | T82337, T67082, R00223, R00329, R16626, R28641, R32574, R51743, R51742, R53339, R53944, R54745, H41976, R91992, R93197, N24801, N94394, W52940, W69738, W69919, AA037409, AA125990, AA188926, AA188971, AA190400, AA226881, AA236272, AA234282, AA216414, AA480990, AA536053, AA576002, AA876410, AA902252, AA988139, N56036, W27433, W27845, AA090944, AA852475, AA912970, AA991732, A1052341, A1088637, A1091099, A1093272, T57777                 |
| 24 | HOSEJ94 | 34 | 795132 | T59457, T77300, R39702, H06484, H06542, H41069, R96192, H89121, H89228, H89121, N21676, N22011, N31083, N71214, N80237, W33046, W38328, AA018173, AA034997, AA035459, AA045662, AA045663, AA046446, AA047840, AA053980, AA056096, AA056157, AA057237, AA058701, AA074821, AA079526,                                                                                                                                                                  |

|    |         |    |        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|----|---------|----|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |         |    |        | AA084570, AA125818, AA125949, AA143149, AA172038, AA172290, AA233777, AA233629                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 26 | HPMFW01 | 36 | 844865 | T78749, R13303, R40786, R40786, H29503, H29504, H29787, N45617, N48825, W81467, W81348, AA074072, AA165298, AA165297, AA426332, AA564851, AA595754, AA604226, AA858439, AA861993, AA946852, AA213464, AA446901, AA598694, AA703596, AA707156, AA679593, AA860722, A1002939                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 27 | HPMSH26 | 37 | 780109 | T65815, T71810, T94336, T94677, T81016, R38719, R62718, R68108, R68156, R68270, R68305, R68787, R68886, R78153, H04747, H04836, H24742, H26582, R96423, H88093, H88094, H88630, H88629, H92610, H96142, H88094, H88630, N21186, N21304, N23613, N23614, N27459, N34816, N63372, N66631, N66793, N67458, N67593, N73666, N78508, N81089, N93723, W07450, W19258, W37482, W37587, W44555, W46637, W47036, W47375, W47581, W48742, W48743, W69372, W69373, W69406, N90911, AA036736, AA037020, AA046206, AA046212, AA046422, AA046434, AA046429, AA055955, AA056182, AA056175, AA055913, AA074728, AA074903, AA079432, AA081840, AA084635, AA088462, AA122208, AA130383, AA131187, AA131123, AA150228, AA167645, AA173792, AA193274, AA235659, AA235965, AA262916, AA459979 |
| 29 | HPWTF53 | 39 | 844737 | AA845804                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 30 | HRTAP63 | 40 | 780698 | T80362, T80478, R26021, R26823, R31829, R37220, R38842, H18682, H18773, H24204, H24217, H24218, H27027, H46491, H47030, R96314, H49227, H49413, H50669, H50762, H85509, H85708, H88357, H88417, H95144, H95180, H88357, N21490, N27003, N35260, N40125, N70494, N77905, N99875, W05840, W25309, W32295, W32702, W32538, W37452, W37577, W37696, W44802, W44808, W44717, W60533, W87586, W87846, AA027857, AA027923, AA033645, AA034476, AA055324, AA083837, AA115539, AA115050, AA125918, AA133758, AA133757, AA152423, AA151732, AA152318, AA150348, AA165032, AA210879, AA214623, AA224004                                                                                                                                                                             |
| 31 | HSSJM47 | 41 | 796096 | R10553                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 32 | HASAU26 | 42 | 845848 | R46537, R50381, H24155, H38325, R83484, H80778, W17012, W52743, W56661, W56553, W59997, W79003, W84895, W84700, AA029081, AA029082, AA037365, AA179393, AA182708, AA427845, AA483287, AA494526, AA513526, AA527305, AA552042, AA563839, AA628070, AA580485, AA658432, AA810917, AA826586, AA829942, AA857237, AA968892, A1015546, A1076886, F18664, W84389, AA651737, AA292010, AA393733, AA397929, F21719, AA704475, AA705524, AA780369, AA853002, AA853001, AA853013, AA853003, A1001734,                                                                                                                                                                                                                                                                              |

|    |         |    |        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|----|---------|----|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |         |    |        | A1078440, A1095487, A1095689, Z25334, AA682646                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 33 | HSIDA33 | 43 | 784543 | R26488, R26510, R84229                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 34 | HSIFO61 | 44 | 845026 | T99761, T99856, R23192, R23191, H17067, H20713, H38876, H52328, AA004615, AA004890, AA086474, AA112729, AA134414, AA134415, AA526905, AA532739, AA570483, AA907899, AA913178, AA913640, AA932997, C03711, C04532, C04547, AA651838, AA481316, AA496348, AA496397, AA496496, AA496621, AA600310, AA705577, AA725306, AA844628, AA846145, AA907827, A1018039                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 35 | HPWTF23 | 45 | 844775 | T60058, T60102, T63278, T63404, T64499, T65926, T65354, T79429, T79517, T27118, R22214, R22268, R34893, R49280, R52727, R49280, H26544, H43597, H44638, R86094, N47563, N47564, N50029, N54684, N54707, N62277, N77565, W46821, W46859, W46870, W58190, W58299, W60279, W60371, W63717, W65468, W65469, W67232, W67342, W72563, W76557, W92333, W92390, AA010680, AA010681, AA018926, AA019007, AA026509, AA040063, AA043646, AA115222, AA115157, AA135362, AA137239, AA147899, AA148642, AA148643, AA190663, AA459957, AA460046, AA464495, AA464496, AA427718, AA429282, AA244422, AA244455, AA279281, AA507838, AA516433, AA523541, AA535070, AA551457, AA602156, AA613662, AA618091, AA576196, AA662310, AA765950, AA862301, AA906753, AA953835, AA953911, AA987730, A1096573, D79925, N85667, C20552, AA147844, AA652629, AA652778, AA292345, AA293158, AA293176, AA292756, AA394163, AA487093, AA599534, AA432382, AA431390, AA679292, AA716187, AA722314, AA775091, AA833692, AA835434, AA909440, AA971998, A1039111, A1074133, A1089512, T24090, D45452, F11656, F11926, F09314, F09572 |
| 36 | HSLHG78 | 46 | 846148 | T60061, R25513, H27055, H28231, H45940, H83457, H83568, N20186, N29088, N46871, N47192, AA148378, AA148413, AA173572, AA173186, AA419059, AA418857, AA418856, AA423815, AA878219, AA902571, AA937546, AA954340, C00626, AA248005, AA724787, AA757232, AA779589, AA884182, A1078276                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 37 | HSSJW30 | 47 | 779822 | T65307, N73284, AA043319, AA043320, AA047422, AA047423                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 38 | HTEDT87 | 48 | 779267 | T75035, R21138, R43650, R46733, R43650, R46733, H16773, H16882, R96518, R96563, N40824, W00616, W46979, W47080, AA027114                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 39 | HTLFI73 | 49 | 846063 | None                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 40 | HTNBJ15 | 50 | 845783 | T90793, R10265, R10264, R21407, R32179, R32228, R68686, R68741, R79633, R79827, H15739, H40220, H40793, R94269, H58305, H58695, H63252, H63329, H78607, H78662,                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

|    |         |    |        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----|---------|----|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |         |    |        | H80484, N34916, N57882, W04585, W31833, W33186, W37252, W68156, AA001417, AA001476, AA044024, AA058969, AA111864, AA149278, AA149304, AA156432, AA251881, AA459353, AA631419, AA669088, AA669116, AA689254, AA730992, AA856892, AA888623, AA976147, AA976169, D80081, N87651, N87946, AA044200, AA083679, AA436984, AA437254, AA633999, AA678955, AA719387, AA860723, AA905274, AA906416, AI051914, AI092296, D20797, Z40781, AA694450                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 41 | HTOET03 | 51 | 845230 | R13210, R49690, R49690, H52976, H63929, N58271, N75539, W05332, AA009639, AA009913, AA232404, AA252672, AA430295, AA252674, AA468736, AA503251, AA523626, AA582115, AA618010, AA580407, AA807231, AA848070, AI052290, AA026033, AA283196, AA443797, AI018643                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 42 | HTXJW06 | 52 | 789201 | T54424, T54472, W56510, W56751, AA057187, AA233545                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 43 | HUKAI28 | 53 | 843240 | R16327, R19012, R41403, R44083, R41403, R44083, AA024569, AA024681, AA514595, AA453472, AA453980, AA909007, Z38629                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 44 | HUKFC71 | 54 | 845161 | H40724, H46968, N42261, W31201, W31772, W74161, AA078878, AA143742, AA147783, AA155778, AA226209, AA226545, AA483925, AA512922, AA618058, AA576035, AA715375, AA740463, AA857299, AA908395, AA936118, F18818, AA488220, AA488254, AA609958, AA722666, AI074905                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 45 | HUKFV37 | 55 | 844644 | T47328, T52071, T71104, T77418, R08599, T96113, T96193, T96194, R01124, T26382, R61783, R72627, R72698, H20537, H20630, H52662, H61205, H62110, N23034, N54144, N93383, N95718, W25402, W37484, W52694, W79486, W79630, W81549, W81550, AA074639, AA458500, AA460236, AA463799, AA282885, AA465145, AA465253, AA468707, AA488766, AA488987, AA525442, AA531066, AA531532, AA536177, AA555138, AA587714, AA588105, AA622357, AA632947, AA635625, AA635797, AA576443, AA577351, AA687321, AA745265, AA832041, AA834496, AA838514, AA856643, AA857987, AA864636, AA872815, AA873160, AA873436, AA878152, AA885559, AA888543, AA916469, AA931570, AA937584, AA961789, AA975671, AI014631, AI028016, AI081520, N83457, AA092490, AA292270, AA394128, AA399135, AA402134, AA402378, AA402983, AA443894, AA456798, AA477555, AA477599, AA609827, AA431853, AA431854, AA704924, AA724420, AA770528, AA897375, AA897757, AA971776, AA973012, AI005213, AI027063, AI075402, AI074246, AI074307, AI086820, AI090476, AI091416, AI096558, D19664 |
| 46 | HUKFY09 | 56 | 784086 | T39302, T40513, T71507, R17294, R51594, R51682, H52242, H52339, N78775, N93954,                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

|    |         |    |        |                                                                                                                          |
|----|---------|----|--------|--------------------------------------------------------------------------------------------------------------------------|
|    |         |    |        | W07057, W40456, AA027305, AA027301,<br>AA027302, AA028141, AA028178, AA057393,<br>AA101174, AA101311, AA129733, AA133347 |
| 47 | HWBBU75 | 57 | 780360 | R84247, H87267, H87322, H92853, W15561                                                                                   |
| 49 | HEMEY47 | 59 | 834491 | R02409, H67089, AA834886, AA873661,<br>AA171479                                                                          |
|    |         |    |        |                                                                                                                          |

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

5

### Examples

#### Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

- Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector.
- 10 Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being
- 15 isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

|    | <u>Vector Used to Construct Library</u> | <u>Corresponding Deposited</u> |
|----|-----------------------------------------|--------------------------------|
|    | <u>Plasmid</u>                          |                                |
|    | Lambda Zap                              | pBluescript (pBS)              |
| 20 | Uni-Zap XR                              | pBluescript (pBS)              |
|    | Zap Express                             | pBK                            |
|    | lafmid BA                               | plafmid BA                     |
|    | pSport1                                 | pSport1                        |
|    | pCMVSPORT 2.0                           | pCMVSPORT 2.0                  |
| 25 | pCMVSPORT 3.0                           | pCMVSPORT 3.0                  |
|    | pCR <sup>®</sup> 2.1                    | pCR <sup>®</sup> 2.1           |



Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Altting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Altting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lacmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention

does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for

bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

- Alternatively, two primers of 17-20 nucleotides derived from both ends of the
- 5 SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is
- 10 1.5-5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and
- 15 the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

- Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods
- 20 include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., *Nucleic Acids Res.* 21(7):1683-1684 (1993).)

- 25 Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A

primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

- 5            This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order
- 10          to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

- This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is
- 15          used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

20          **Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

25          **Example 3: Tissue Distribution of Polypeptide**

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with  $P^{32}$  using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

- 10 Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films
- 15 developed according to standard procedures.

#### **Example 4: Chromosomal Mapping of the Polynucleotides**

- An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This
- 20 primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C.
- Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios,
- 25 Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose

gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

**Example 5: Bacterial Expression of a Polypeptide**

5           A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product  
10       into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning  
15       sites.

          The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which  
20       expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

          Clones containing the desired constructs are grown overnight (O/N) in liquid  
25       culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The

cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

- 5           Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from  
10   QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

- Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then  
15   washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

- The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-  
20   NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50  
25   mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains:

- 1) a neomycinphosphotransferase gene as a selection marker, 2) an *E. coli* origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacI*q). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.
- 10 DNA can be inserted into the pHEa by restricting the vector with *NdeI* and *XbaI*, *BamHI*, *XhoI*, or *Asp718*, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for *NdeI* (5' primer) and *XbaI*, *BamHI*, *XhoI*, or
- 15 *Asp718* (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### 20 **Example 6: Purification of a Polypeptide from an Inclusion Body**

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

- Upon completion of the production phase of the *E. coli* fermentation, the cell
- 25 culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield



of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

5           The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

10           The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

          Following high speed centrifugation (30,000 xg) to remove insoluble particles,  
15   the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

          To clarify the refolded polypeptide solution, a previously prepared tangential  
20   filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a  
25   stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

#### **Example 7: Cloning and Expression of a Polypeptide in a Baculovirus**

##### **Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak

Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

5 Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-10 39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does 15 not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

20 The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and 25 optionally, can be dephosphorylated using calf intestinal phosphatase, using routine

procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue

- 5 (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

- 10 Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a
- 15 microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is
- 20 then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

- After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life
- 25 Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of

a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

#### **Example 8: Expression of a Polypeptide in Mammalian Cells**

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and

signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV1, HIV1 and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers,

the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

- 5 Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g.,
- 10 with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

- Specifically, the plasmid pC6, for example, is digested with appropriate
- 15 restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

- A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide.
- 20 Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

- The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1%
- 25 agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 a pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200  $\mu$ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

#### **Example 9: Protein Fusions**

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG



domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACACACATGCCACCGTGC  
 CCAGCACCTGAATTCGAGGGTGACCGTCAGTCTTCTCTCCCCCAAAA  
 5 CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGT  
 GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG  
 ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTA  
 CAACAGCACGTACCGTGTGGTCAGCGTCCTACCGTCTGCACCAGGACT  
 GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA  
 10 ACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC  
 CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG  
 GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT  
 GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACATAAGACCACGCCT  
 CCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTG  
 15 GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGTCCGTGATGCA  
 TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG  
 GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

**Example 10: Production of an Antibody from a Polypeptide**

20 The antibodies of the present invention can be prepared by a variety of  
 methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells  
 expressing a polypeptide of the present invention is administered to an animal to  
 induce the production of sera containing polyclonal antibodies. In a preferred  
 method, a preparation of the secreted protein is prepared and purified to render it  
 25 substantially free of natural contaminants. Such a preparation is then introduced into  
 an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., *Nature* 256:495 (1975); Köhler et al., *Eur. J. Immunol.* 6:511 (1976); Köhler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells,

and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of  
5 further protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub>  
10 fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced  
15 using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson  
20 et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

#### **Example 11: Production Of Secreted Protein For High-Throughput Screening**

##### **Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described in Examples 13-20.

- First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution
- 5 (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipettor may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered
- 10 Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

- Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x
- 15 Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

- The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods
- 20 described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be
- 25 transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates  
 5 off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1% BSA in  
 10 DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl<sub>2</sub> (anhyd); 0.00130 mg/L CuSO<sub>4</sub>·5H<sub>2</sub>O; 0.050 mg/L of Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O; 0.417 mg/L of FeSO<sub>4</sub>·7H<sub>2</sub>O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl<sub>2</sub>; 48.84 mg/L of MgSO<sub>4</sub>; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO<sub>3</sub>; 62.50 mg/L of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O; 71.02 mg/L of Na<sub>2</sub>HPO<sub>4</sub>; 4320 mg/L of ZnSO<sub>4</sub>·7H<sub>2</sub>O; .002 mg/L of Arachidonic Acid ; 1.022 mg/L  
 15 of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml  
 20 of L-Asparagine-H<sub>2</sub>O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H<sub>2</sub>O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H<sub>2</sub>O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0  
 25 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H<sub>2</sub>O; 99.65 mg/ml of L-

Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and

5 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed

10 with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end

15 of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants

20 from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the

25 invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

**Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the  
5 Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six  
10 members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in  
15 tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are  
20 generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-  
25 12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b)



Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

- Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn
- 5    activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

- Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines
- 10   are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

|    |                                  |             | <u>JAKs</u> |             |             | <u>STATs</u> | <u>GAS(elements) or ISRE</u> |
|----|----------------------------------|-------------|-------------|-------------|-------------|--------------|------------------------------|
|    | <u>Ligand</u>                    | <u>tyk2</u> | <u>Jak1</u> | <u>Jak2</u> | <u>Jak3</u> |              |                              |
|    | <u>IFN family</u>                |             |             |             |             |              |                              |
| 5  | IFN-a/B                          | +           | +           | -           | -           | 1,2,3        | ISRE                         |
|    | IFN-g                            |             | +           | +           | -           | 1            | GAS (IRF1>Lys6>IFP)          |
|    | Il-10                            | +           | ?           | ?           | -           | 1,3          |                              |
|    | <u>gp130 family</u>              |             |             |             |             |              |                              |
| 10 | IL-6 (Pleiotrophic)              | +           | +           | +           | ?           | 1,3          | GAS (IRF1>Lys6>IFP)          |
|    | Il-11(Pleiotrophic)              | ?           | +           | ?           | ?           | 1,3          |                              |
|    | OnM(Pleiotrophic)                | ?           | +           | +           | ?           | 1,3          |                              |
|    | LIF(Pleiotrophic)                | ?           | +           | +           | ?           | 1,3          |                              |
|    | CNTF(Pleiotrophic)               | -/+         | +           | +           | ?           | 1,3          |                              |
| 15 | G-CSF(Pleiotrophic)              | ?           | +           | ?           | ?           | 1,3          |                              |
|    | IL-12(Pleiotrophic)              | +           | -           | +           | +           | 1,3          |                              |
|    | <u>g-C family</u>                |             |             |             |             |              |                              |
|    | IL-2 (lymphocytes)               | -           | +           | -           | +           | 1,3,5        | GAS                          |
| 20 | IL-4 (lymph/myeloid)             | -           | +           | -           | +           | 6            | GAS (IRF1 = IFP >>Ly6)(IgH)  |
|    | IL-7 (lymphocytes)               | -           | +           | -           | +           | 5            | GAS                          |
|    | IL-9 (lymphocytes)               | -           | +           | -           | +           | 5            | GAS                          |
|    | IL-13 (lymphocyte)               | -           | +           | ?           | ?           | 6            | GAS                          |
|    | IL-15                            | ?           | +           | ?           | +           | 5            | GAS                          |
| 25 | <u>gp140 family</u>              |             |             |             |             |              |                              |
|    | IL-3 (myeloid)                   | -           | -           | +           | -           | 5            | GAS (IRF1>IFP>>Ly6)          |
|    | IL-5 (myeloid)                   | -           | -           | +           | -           | 5            | GAS                          |
|    | GM-CSF (myeloid)                 | -           | -           | +           | -           | 5            | GAS                          |
| 30 | <u>Growth hormone family</u>     |             |             |             |             |              |                              |
|    | GH                               | ?           | -           | +           | -           | 5            |                              |
|    | PRL                              | ?           | +/-         | +           | -           | 1,3,5        |                              |
|    | EPO                              | ?           | -           | +           | -           | 5            | GAS(B-CAS>IRF1=IFP>>Ly6)     |
| 35 | <u>Receptor Tyrosine Kinases</u> |             |             |             |             |              |                              |
|    | EGF                              | ?           | +           | +           | -           | 1,3          | GAS (IRF1)                   |
|    | PDGF                             | ?           | +           | +           | -           | 1,3          |                              |

|       |   |   |   |   |     |                |
|-------|---|---|---|---|-----|----------------|
| CSF-1 | ? | + | + | - | 1,3 | GAS (not IRF1) |
|-------|---|---|---|---|-----|----------------|

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAAGTCCCGCCCCTAACTCCGCCATCCCGCCCCTAACTCCGCCAGTTCGCCCATTTCTCCGCCCCATGGCTGACTAATTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCCTTTTGCAAAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter

5 molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and

10 XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-

15 SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding

20 as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and

16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be

25 substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-

2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

5    **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP  
10    activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate  
15    stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing  
20    concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in  
25    RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life

Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

- During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final
- 5 concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

- The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with
- 10 supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

- On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being
- 15 screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

- Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

- 20 After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

- 25 The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul

samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve  
5 as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as,  
10 stable transfected cells, which would be apparent to those of skill in the art.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells.

15 Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct  
20 produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

25 Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM



KCl, 375  $\mu$ M  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675  $\mu$ M  $\text{CaCl}_2$ . Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

- 5        The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400  $\mu$ g/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400  $\mu$ g/ml G418 for couple of passages.

- These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described  
10    growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200  $\mu$ l cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

- Add 50  $\mu$ l of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml  
interferon gamma can be used which is known to activate U937 cells. Over 30 fold  
15    induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

**Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

- When cells undergo differentiation and proliferation, a group of genes are  
20    activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

- Particularly, the following protocol is used to assess neuronal activity in PC12  
25    cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl

phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

- 5       The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

- 5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)  
10       5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

- Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1  
15       promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

- 20       PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and  
25       resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

20

**Example 16: High-Throughput Screening Assay for T-cell Activity**

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of

25

apoptosis (NF- $\kappa$ B appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

- In non-stimulated conditions, NF- $\kappa$ B is retained in the cytoplasm with I- $\kappa$ B (Inhibitor  $\kappa$ B). However, upon stimulation, I- $\kappa$ B is phosphorylated and degraded, causing NF- $\kappa$ B to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- $\kappa$ B include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

- Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF- $\kappa$ B promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF- $\kappa$ B would be useful in treating diseases. For example, inhibitors of NF- $\kappa$ B could be used to treat those diseases related to the acute or chronic activation of NF- $\kappa$ B, such as rheumatoid arthritis.

- To construct a vector containing the NF- $\kappa$ B promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF- $\kappa$ B binding site (GGGGACTTCCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

- 5':GCGGCCTCGAGGGGACTTCCCGGGGACITTCGGGGACITTCGGGGAC  
TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTGTCAAAGCCTAGGC:3' (SEQ ID NO:4)

- PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCAGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCC  
 5 ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCC  
 ATCCCGCCCCTAACTCCGCCCAGTTCGCCCATTCTCCGCCCCATGGCTGA  
 CTAATTTTTTTTATTTATGTCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTA  
 TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCCTTTTGCAAAAA  
 GCTT:3' (SEQ ID NO:10)

10

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

15 In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette, was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

20 Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

25

#### **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

- 5 Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

- Cool the samples to room temperature for 15 minutes. Empty the dispenser
- 10 and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at
- 15 each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

**Reaction Buffer Formulation:**

| # of plates | Rxn buffer diluent (ml) | CSPD (ml) |
|-------------|-------------------------|-----------|
| 10          | 60                      | 3         |
| 11          | 65                      | 3.25      |
| 12          | 70                      | 3.5       |
| 13          | 75                      | 3.75      |
| 14          | 80                      | 4         |
| 15          | 85                      | 4.25      |
| 16          | 90                      | 4.5       |
| 17          | 95                      | 4.75      |
| 18          | 100                     | 5         |
| 19          | 105                     | 5.25      |
| 20          | 110                     | 5.5       |

|    |     |       |
|----|-----|-------|
| 21 | 115 | 5.75  |
| 22 | 120 | 6     |
| 23 | 125 | 6.25  |
| 24 | 130 | 6.5   |
| 25 | 135 | 6.75  |
| 26 | 140 | 7     |
| 27 | 145 | 7.25  |
| 28 | 150 | 7.5   |
| 29 | 155 | 7.75  |
| 30 | 160 | 8     |
| 31 | 165 | 8.25  |
| 32 | 170 | 8.5   |
| 33 | 175 | 8.75  |
| 34 | 180 | 9     |
| 35 | 185 | 9.25  |
| 36 | 190 | 9.5   |
| 37 | 195 | 9.75  |
| 38 | 200 | 10    |
| 39 | 205 | 10.25 |
| 40 | 210 | 10.5  |
| 41 | 215 | 10.75 |
| 42 | 220 | 11    |
| 43 | 225 | 11.25 |
| 44 | 230 | 11.5  |
| 45 | 235 | 11.75 |
| 46 | 240 | 12    |
| 47 | 245 | 12.25 |
| 48 | 250 | 12.5  |
| 49 | 255 | 12.75 |
| 50 | 260 | 13    |

---

**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes

in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to  $2.5 \times 10^6$  cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to  $1 \times 10^6$  cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.



- To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an
- 5 extracellular signaling event which has resulted in an increase in the intracellular  $Ca^{++}$  concentration.

**Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

- 10 The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is
- 15 unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

- Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include
- 20 receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

- Because of the wide range of known factors capable of stimulating tyrosine
- 25 kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the

following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

- Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from
- 5 Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or
- 10 calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen
- 15 Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

- To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20
- 20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is
- 25 shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane

bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4  
5 degrees C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by  
10 determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

15 The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2</sub><sup>+</sup> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the  
20 components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction  
25 mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20

min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

#### **Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against

Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

- 5           A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.
- 10           After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard
- 15           procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

20    **Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide**

- RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA
- 25    is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for

30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical

Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

5

**Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample**

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide  
10 is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with  
15 specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample  
20 containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature.  
25 The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

### **Example 23: Formulation**

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01



mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable

polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

- 5           Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., *Id.*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

- Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see generally*, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317-327 and 353-365 (1989)).
- 15       Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small
- 20       (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

- In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see Langer, supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar

alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will  
5 be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container  
10 having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml  
15 vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the  
20 invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

25 The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the

Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Viroosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either

concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through  
5 separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like  
10 molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No.  
15 WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB, TR2 (International  
20 Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International  
Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms  
25 CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™,

KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment,



Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered  
5 in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered  
10 with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-  
15 sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other  
20 immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™  
25 (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific

embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations.

- 5 Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation  
10 therapy (e.g., bone marrow transplant).

- In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-  
15 inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazon, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal,  
20 pifoxime, proquazone, proxazole, and tenidap.

- In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to,  
25 antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-

FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number

5 EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., *Growth Factors*, 4:259-268 (1993);

10 Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth

15 Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent

20 Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention

25 include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, 5 FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

10 **Example 24: Method of Treating Decreased Levels of the Polypeptide**

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the 15 invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering 20 to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on 25 administration and formulation, are provided in Example 23.

**Example 25: Method of Treating Increased Levels of the Polypeptide**

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising  
5 administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of  
10 decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation  
15 of the antisense polynucleotide is provided in Example 23.

**Example 26: Method of Treatment Using Gene Therapy-Ex Vivo**

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a  
20 subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed  
25 to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS,

penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge.

- 5 The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

- 10 The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine
- 15 sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene
- 20 of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector.

- 25 The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

**Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention**

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.



Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the

polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary  
5 phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub> HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the  
10 supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately  $3 \times 10^6$  cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to  
15 construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an  
20 XbaI site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-  
25 digested pUC18 plasmid.

- Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120  $\mu\text{g/ml}$ . 0.5 ml of the cell suspension (containing approximately  $1.5 \times 10^6$  cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is
- 5 performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960  $\mu\text{F}$  and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.
- 10 Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a
- 15 further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

20

#### **Example 28: Method of Treatment Using Gene Therapy - In Vivo**

- Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA)
- 25 sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter

or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., *Cardiovasc. Res.* 35(3):470-479 (1997); Chao et al., 5 *Pharmacol. Res.* 35(6):517-522 (1997); Wolff, *Neuromuscul. Disord.* 7(5):314-318 (1997); Schwartz et al., *Gene Ther.* 3(5):405-411 (1996); Tsurumi et al., *Circulation* 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial 10 space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the 15 cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) *Ann. NY Acad. Sci.* 772:126-139 and Abdallah B. et al. (1995) *Biol. Cell* 85(1):1-7) which can be prepared by methods well known to those 20 skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies 25 techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies

have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the

condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or  
5 mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard  
10 recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on  
15 the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

20 After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15  $\mu$ m cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA  
25 in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice.

The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

5     **Example 29: Transgenic Animals.**

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a  
10    specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (*i.e.*, polynucleotides of the invention) into animals to produce the founder lines of  
15    transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci.,  
20    USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, *e.g.*, Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent  
25    stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of

such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into  
5 enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their  
10 cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory  
15 sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the  
20 endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science  
25 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific



inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening  
5 may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse  
10 transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding  
15 strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given  
20 integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

25 Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of

polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

5    **Example 30: Knock-Out Animals.**

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by  
10   reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express  
15   polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be  
20   used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

25       In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically

engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For

example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

- Transgenic and "knock-out" animals of the invention have uses which include,
- 5 but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

10 **Example 31: Production of an Antibody**

a) Hybridoma Technology

- The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing XXX are administered to an animal to induce the production of sera
- 15 containing polyclonal antibodies. In a preferred method, a preparation of XXX protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

- Monoclonal antibodies specific for protein XXX are prepared using
- 20 hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with XXX polypeptide or, more preferably, with a secreted XXX polypeptide-expressing cell.
- 25 Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine

serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the XXX polypeptide.

Alternatively, additional antibodies capable of binding to XXX polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the XXX protein-specific antibody can be blocked by XXX. Such antibodies comprise anti-idiotypic antibodies to the XXX protein-specific antibody and are used to immunize an animal to induce formation of further XXX protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein.

(See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed

Against XXX From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against XXX to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10<sup>9</sup> E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody

fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10<sup>13</sup> transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10<sup>13</sup> TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification

with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect *E. coli* HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

**Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation**

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective



ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with  $^3$ H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peria-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

5

### **Example 33: T Cell Proliferation Assay**

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of  $^3\text{H}$ -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100  $\mu\text{l}$ /well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1  $\mu\text{g}/\text{ml}$  in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5  $\times 10^4$ /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of polypeptides of the invention (total volume 200  $\mu\text{l}$ ). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100  $\mu\text{l}$  of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100  $\mu\text{l}$  of medium containing 0.5  $\mu\text{Ci}$  of  $^3\text{H}$ -thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of polypeptides of the invention.

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to

25

test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

**Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells**

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCyRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

**Effect on the production of cytokines.** Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to

measure the IL-12 release as follows. Dendritic cells ( $10^6/\text{ml}$ ) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or

activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque  
5 gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results  
10 from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative  
15 control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before FACSscan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

20

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml  
25 with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the

cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20  $\mu$ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of  $H_2O_2$  produced by the macrophages, a standard curve of a  $H_2O_2$  solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 35: Biological Effects of Polypeptides of the Invention**

#### Astrocyte and Neuronal Assays:

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite

outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

#### Fibroblast and endothelial cell assays:

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA



medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

#### Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity

reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

5 It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J.  
10 Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential  
15 effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium  
20 containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

25 Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are

proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

5           The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

10       **Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells**

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2.5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16  
15   units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is  
20   determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to  
25   test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells**

- 5           For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight.
- 10          After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF<sub>165</sub> or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is
- 15          subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

- The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the
- 20          invention.

**Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect**

- 25          HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6

mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After  
5 counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV  
10 fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
15 invention.

#### **Example 39: Stimulation of Endothelial Migration**

This example will be used to explore the possibility that a polypeptide of the  
20 invention may stimulate lymphatic endothelial cell migration.

Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours  
25 at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25

ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber,  $2.5 \times 10^5$  cells suspended in 50  $\mu$ l M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO<sub>2</sub> to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

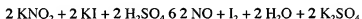
#### **Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells**

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after

reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of  $\text{KNO}_2$  (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and  $\text{H}_2\text{SO}_4$ . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C.

The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 41: Effect of Polypeptides of the Invention on Cord Formation in Angiogenesis**

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200  $\mu$ l/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300  $\mu$ l Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.



**Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane**

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old quail embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse**

*In vivo* angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine

extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 44: Rescue of Ischemia in Rabbit Lower Limb Model**

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one

femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al.* *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al.* *Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al.* *J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

**Example 45: Effect of Polypeptides of the Invention on Vasodilation**

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as  $p < 0.05$  vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 46: Rat Ischemic Skin Flap Model**

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

- a) Ischemic skin
- b) Ischemic skin wounds
- c) Normal wounds

The experimental protocol includes:

a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

c) Topical treatment with a polypeptide of the invention of the excisional wounds  
5 (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to  
10 test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 47: Peripheral Arterial Disease Model**

15 Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

20 b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

c) The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention  
25 and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

**Example 48: Ischemic Myocardial Disease Model**

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after  
10 coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is  
15 delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

The studies described in this example tested activity of a polypeptide of the  
20 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 49: Rat Corneal Wound Healing Model**

25

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

- a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.
- 5 b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.
- c) Making a pocket (its base is 1-1.5 mm from the edge of the eye).
- d) Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.
- 10 e) Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
15 invention.

**Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models**

20 A. *Diabetic db+/db+ Mouse Model.*

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is  
25 dependent on formation of granulation tissue and re-epithelialization rather than

contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal  
5 heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al. Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375  
10 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*,  
15 *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of*  
20 *Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All  
25 manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional



Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of  
5 wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following  
10 wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of  
15 surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses,  
20 from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral  
25 buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by  
5 establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

10

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the  
15 healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

20 Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

25 Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon

cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

### ***B. Steroid Impaired Rat Model***

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *Am. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

5           Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad*  
10 *libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

          The wounding protocol is followed according to section A, above. On the day of  
15 wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the  
20 testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

          Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement  
25 on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a

calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups  
5 received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

10 Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing  
15 the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

20

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the  
25 healing process and the morphologic appearance of the repaired skin is improved by

treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

- 5           The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

10   **Example 51: Lymphedema Animal Model**

- or       The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system
- 15   in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

- Prior to beginning surgery, blood sample is drawn for protein concentration
- 20   analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The
- 25   intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's

Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

- 5           Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema  
10 software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca<sup>2+</sup> comparison.

- 15           Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

- 20           Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

- 25           The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.



**Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF- $\alpha$ ), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$  treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently

washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

- 5 Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90  $\mu$ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are
- 10 aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

- Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-
- 15 1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

- Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with
- 20 PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5} > 10^{-2}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is
- 25 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M

NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation**

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low

level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL-3, and then contacted with the compound that is

5 being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one  
10 hour. The cell count is adjusted to  $2.5 \times 10^5$  cells/ml. During this time, 100  $\mu$ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one  
15 hour, 10  $\mu$ l of prepared cytokines, 50  $\mu$ l SID (supernatants at 1:2 dilution = 50  $\mu$ l) and 20  $\mu$ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100  $\mu$ l. The plates are then placed in a 37°C/5% CO<sub>2</sub> incubator for five days.

Eighteen hours before the assay is harvested, 0.5  $\mu$ Ci/well of [3H] Thymidine  
20 is added in a 10  $\mu$ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60  $\mu$ l Microscint is added to each well and the plate sealed with  
25 TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined

via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

- The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.
- 10 In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

- The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

**Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECR)**

- 20 The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

- Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM.

Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the  $\alpha_5\beta_1$  and  $\alpha_4\beta_1$  integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of  $0.2 \mu\text{g}/\text{cm}^2$ . Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5%  $\text{CO}_2$ , 7%  $\text{O}_2$ , and 88%  $\text{N}_2$ ) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA.

Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACSscan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular gene product is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

**Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation**

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-

assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF $\alpha$  stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100  $\mu$ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5  $\mu$ g/ml hEGF, 5mg/ml insulin, 1 $\mu$ g/ml hFGF, 50mg/ml gentamycin, 50  $\mu$ g/ml Amphotericin B, 5%FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50 $\mu$ g/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNF $\alpha$  is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO $_2$  until day 5.



- Transfer 60 $\mu$ l from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100  $\mu$ l in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10 $\mu$ l). Return plates to incubator for 3 to 4 hours. Then measure
- 5 fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100  $\mu$ l/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

- 10 On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200  $\mu$ l/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50  $\mu$ l/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make
- 15 dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100  $\mu$ l/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

- 20 Add 100  $\mu$ l/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

- A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or
- 25 smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product

of interest. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

5    **Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in  
10    both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local  
15    vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is  
20    removed from the cells and replaced with 100  $\mu$ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca++  
25    and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA

and drained. 10  $\mu$ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 ( $10^6$ ) >  $10^{0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

20

#### **Example 57: Alamar Blue Endothelial Cells Proliferation Assay**

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue

Proliferation Assay is prepared in EGM-2MV with 10 ng/ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration.

- Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free  
5 medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

- Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37°C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with  
10 GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37°C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to  
15 each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

- Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell  
20 growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional  
25 to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the

output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

**Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction**

5           This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine  
10       production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

          Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These  
15       include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

20       Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM<sup>®</sup>, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to  $2 \times 10^6$  cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is  
25       adjusted to  $2 \times 10^5$  cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50  $\mu$ l) is

added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 µg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO<sub>2</sub>, and 1 µCi of [<sup>3</sup>H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Additionally, U.S. provisional application Serial No. 60/138,628 is hereby incorporated by reference in its entirety.

|                                              |          |                                                    |            |
|----------------------------------------------|----------|----------------------------------------------------|------------|
| Applicant's or agent's file reference number | PS552PCT | International application No. <u>75 100 749 21</u> | UNASSIGNED |
|----------------------------------------------|----------|----------------------------------------------------|------------|

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                            |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| A. The indications made below relate to the microorganism referred to in the description<br>on page <u>130</u> , line <u>N/A</u>                                                                                                                                                                                                                                                                                                                                                     |                            |
| B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                         |                            |
| Name of depositary institution American Type Culture Collection                                                                                                                                                                                                                                                                                                                                                                                                                      |                            |
| Address of depositary institution (including postal code and country)<br>10801 University Boulevard<br>Manassas, Virginia 20110-2209<br>United States of America                                                                                                                                                                                                                                                                                                                     |                            |
| Date of deposit<br>29 April 1999                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Accession Number<br>203979 |
| C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                              |                            |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                            |
| D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)                                                                                                                                                                                                                                                                                                                                                                           |                            |
| Europe<br>In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).<br>Continued on the Attached Pages 2 & 3 |                            |
| E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)                                                                                                                                                                                                                                                                                                                                                                                                                |                            |
| The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")                                                                                                                                                                                                                                                                                                                 |                            |

|                                                                                                                                                |                                                                                                            |
|------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| For receiving Office use only                                                                                                                  | For International Bureau use only                                                                          |
| <input checked="" type="checkbox"/> This sheet was received with the international application<br><i>Marlene J. Gump</i><br>Authorized officer | <input type="checkbox"/> This sheet was received by the International Bureau on:<br><br>Authorized officer |



**ATCC Deposit No. 203979****Page No. 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 203979**  
**Page No. 3**

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group
- 5 consisting of:
- (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a
  - 10 polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - 15 (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X,
  - 20 having biological activity;
  - (f) a polynucleotide which is a variant of SEQ ID NO:X;
  - (g) a polynucleotide which is an allelic variant of SEQ ID NO:X;
  - (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;

(i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

5

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

10

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

15

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

20

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

25

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.
- 5 8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.
9. A recombinant host cell produced by the method of claim 8.
- 10 10. The recombinant host cell of claim 9 comprising vector sequences.
11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:
- (a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence
- 15 included in ATCC Deposit No:Z;
- (b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;
- (c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- 20 (d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- (e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- (f) a full length protein of SEQ ID NO:Y or the encoded sequence included in
- 25 ATCC Deposit No:Z;
- (g) a variant of SEQ ID NO:Y;

- (h) an allelic variant of SEQ ID NO:Y; or
- (i) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

- (a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and
- (b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

5

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

10 (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

15 (a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

20

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

25 (c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.



<110> Human Genome Sciences, Inc.

<120> 49 Human Secreted Proteins

<130> PS552PCT

<140> Unassigned

<141> 2000-06-01

<150> 60/138,628

<151> 1999-06-11

<160> 186

<170> PatentIn Ver. 2.0

<210> 1

<211> 733

<212> DNA

<213> Homo sapiens

<400> 1

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| gggatccgga | gcccaaatct | tctgacaaaa | ctcacacatg | cccaccgtag | ccagcacctg | 60  |
| aattcgaggg | tgcacggtca | gtcttctctt | tccccccaaa | acccaaggac | accctcatga | 120 |
| tctcccgagc | tcttgaggtc | acatgcgttg | tggtagcgtg | aagccacgaa | gaccctgagg | 180 |
| tcaagttcaa | ctggtagctg | gacggcggtg | aggtgcataa | tgccaagaca | aagccgcggg | 240 |
| aggagcagta | caacagcacg | taccgtgttg | tcagcgtctt | caccgtctct | caccaggact | 300 |
| ggctgaatgg | caaggagtac | aagtgcgaag | tctccaacaa | agcctctcca | acccccatcg | 360 |
| agaaaacat  | ctccaaagcc | aaagggcagc | cccagagaac | acaggtgtac | accctgcccc | 420 |
| catcccgga  | tgagctgacc | aagaaccagg | tcagcctgac | ctgcctggtc | aaaggcttct | 480 |
| atccaaagca | catcgccgtg | gagtgaggga | gcaatgggga | gccggagaac | aactacaaga | 540 |
| ccacgcctcc | cgtgctggac | tccgacggct | ccttcttctt | ctacagcaag | ctcacccgtg | 600 |
| acaagagcag | gtggcagcag | gggaacgtct | tctcatgtct | cgtgatgcat | gaggctctgc | 660 |
| acaaccacta | cacgcagaag | agcctctccc | tgtctccggg | taaatgagtg | cgacggccgc | 720 |
| gactctagag | gat        |            |            |            |            | 733 |

<210> 2

<211> 5

<212> PRT

<213> Homo sapiens

<220>

<221> Site

<222> (3)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 2

Trp Ser Xaa Trp Ser

1 5

<210> 3

<211> 86

<212> DNA

<213> Homo sapiens

<400> 3  
 gcgcctcgcag atttccccga aatctagatt tccccgaaat gatttccccg aaatgatttc 60  
 cccgaaatat ctgccatctc aattag 86

<210> 4  
 <211> 27  
 <212> DNA  
 <213> Homo sapiens

<400> 4  
 gcggcgaagct ttttgcaaaag cctaggc 27

<210> 5  
 <211> 271  
 <212> DNA  
 <213> Homo sapiens

<400> 5  
 ctgcagattt ccccgaaatc tagatttccc cgaaatgatt tccccgaaat gatttccccg 60  
 aaatatctgc catctcaatt agtcagcaac catagtcccg cccctaactc cgcccatccc 120  
 gcccttaact ccgcccattt ccgcccattt tccgcccatt ggctgactaa ttttttttat 180  
 ttatgcagag gccgaggccg cctcggcctc tgagctatct cagaagtagt gaggaggctt 240  
 ttttgagggc ctaggctttt gcaaaaaagct t 271

<210> 6  
 <211> 32  
 <212> DNA  
 <213> Homo sapiens

<400> 6  
 gcgctcgagg gatgacagcg atagaacccc gg 32

<210> 7  
 <211> 31  
 <212> DNA  
 <213> Homo sapiens

<400> 7  
 gcgaagcttc gcgactcccc ggatccgcct c 31

<210> 8  
 <211> 12  
 <212> DNA  
 <213> Homo sapiens

<400> 8  
 ggggactttc cc 12

<210> 9  
 <211> 73  
 <212> DNA  
 <213> Homo sapiens

<400> 9  
 gcggcctcga ggggactttc cgggggactt tccggggact ttccggggact ttccatcctg 60  
 ccatctcaat tag 73

<210> 10  
 <211> 256  
 <212> DNA  
 <213> Homo sapiens

<400> 10  
 ctgaggggga ctttcccggg gactttccgg ggaactttccg ggactttcca tctgccatct 60  
 caattagatca gcaaccatag tcccgccctt aactccgccc atcccgcccc taactccgcc 120  
 cagttccgcc cattctccgc cccatggctg actaattttt tttatttatg cagaggccga 180  
 ggccgctcgc gcctctgagc tattccagaa gtagtgagga ggcatttttg gaggcctagg 240  
 cttttgcaaa aaagctt 256

<210> 11  
 <211> 1887  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (1882)  
 <223> n equals a,t,g, or c

<400> 11  
 gtccatctta gtcaactcta tgccacctct tcttctctca gtccctccac ctgatggctc 60  
 cgacacttca tcactccacca cctcctggag ggggtaccct gagggtgctcc gctgggggct 120  
 ccgctctttc tggggctgcg gttgatggct catcatgac tttcccaaaa tctgtcccat 180  
 ctccaccaac ctagtctctg ttctgtcctt ggtctcttct tggacactgc tgggattccag 240  
 aagagtgtgt tatcaattct cgaggctggg agaagtcagg agtgaggaaac agctctgaga 300  
 agttactgtg gtcccaactga actccagggt gccgacagag tccggtccct ccaatcagga 360  
 aggtcggaat ctctgatgtc atcgctcatg ccaacctggc aaccagtttg aaaaaaaa 420  
 catgtaactg ccaggctgat ctcttgtcct ggagatcctg ggtgaatggt atctctgcc 480  
 actgtcccaa cctcagacca ytggtccaaa gcattctcag ggwtccrca tccctctrtt 540  
 cccctgcccc gcagaggctg tgtcctctcc actcaaaagc tgaagcrtgt tggggctctcy 600  
 tcttctctgt acatgcccggt ttcagagtc agtctgggtg gagagggatc aggatgggaa 660  
 agaaaagttag ggtaagcaga aacgatgaaa ccttacaaga gtgagattat catgtacaag 720  
 agatcccaag aacattgact tgatgaaaaa gtccacatcag agcaactcaat ttggcagagg 780  
 ttttctgcgc agtgcttact gacattcact gtccgagatt ctgtactggg ggtcacgcgc 840  
 tctctgcgcc taaggcatct ttgagtccaa gagatatttt gaggactgga aatcatagga 900  
 aactgcccat gagttcacac atatttccaa tgggtgtccc aatttccagg agtccacgga 960  
 tcacctaaaag ccagcccctc cagtttggct agaaaaactct atatatcaag ttttgtatca 1020  
 tatgtattgc tcttaactca gaaaattcca ccatttatag cagtgggtta tttatttata 1080  
 ccattgaagg aaatgggtta tttatgaatc tatattatgg atattctata agatactggg 1140  
 tgtacaaaaa gactaagtcg aaaaactctca gctgtgcaca gtggctcatg yttgttaaycc 1200  
 catctctttg ggtgscacag ggagggaagac tgcctgaggc cagcagttca agacaggtat 1260  
 aggcacacata gcaagagccc atctctaaaa caaaacaaaa caaaattagc 1320  
 cagggtgctg ggtgtgcacc tgtgttccaa caacttgaga gactgaggtg gcaggaggat 1380  
 tggctgagcc taggagttag gggctgcagt gagctgtgat cgtgacacgc cactccagtc 1440  
 ctggcaacac agcaagrcct tgtgtcaaaa aaattttttt aatttaaatat aaaagagttt 1500  
 catgacattc agagaccatc caaagaacct gtgggttccg gccaggcaca gtgctcacgc 1560  
 ctgtaatccc agccctttgg gaggccatag cagggtggtc gcttgaggtc aggagtttaa 1620  
 gagcagccgt gccaacatgg tgaacccca tctcttctaa aaatacaaaa aattagtcag 1680

|             |             |            |            |             |            |      |
|-------------|-------------|------------|------------|-------------|------------|------|
| gcattggtggt | gggtgctgct  | aatcccagcc | actcaggagg | cgggggacagc | agaatgctct | 1740 |
| aaactctggga | ggcggcagggt | gcagtgagcc | aaggtcacac | cattgcactc  | cagcctgggc | 1800 |
| aacaagagca  | aaactcacatc | tcaaaaaaaa | ctcagggggg | ggcccggtac  |            | 1860 |
| ccaattcgcc  | ctatggtgag  | tngaattg   |            |             |            | 1887 |

<210> 12  
 <211> 2285  
 <212> DNA  
 <213> Homo sapiens

|            |             |             |            |             |            |      |
|------------|-------------|-------------|------------|-------------|------------|------|
| <400> 12   |             |             |            |             |            |      |
| tgtctctgac | cacggctgta  | gaggtggccc  | agcgtggcg  | rgagctggct  | gagaagctgg | 60   |
| ccarggtctc | caagcagcag  | atggacgcct  | acgagtcctc | ccaccgggac  | aggaacgggg | 120  |
| tttgtgacag | cgaggccatg  | tggaaagcctg | cgtatgactt | cttactcacc  | tggagccatc | 180  |
| agatcgggga | cagctacggc  | gatgtcatcc  | aggagctgca | cctgggacctg | gacaagatga | 240  |
| aaaaccccat | caccaagcgc  | tggaaacacc  | tcactgggac | tctgatcttg  | gtgaactccc | 300  |
| tggagctttc | gagagcagcc  | gccttcagcc  | ctgaggacca | ggagcacttc  | gtgatttgaa | 360  |
| tgggtccctc | cccctccctg  | tgcctggag   | tgcagccctc | cttctgccct  | cgctgccctg | 420  |
| ctgtcacccg | ggagctgaag  | agggaggaag  | ggcgggctgc | tcagacagat  | ttagggcccg | 480  |
| ccagctaggc | tacacccatc  | atgcgcgcgc  | ctcctccatc | gagggagagg  | cctgaaggga | 540  |
| ctgcctactg | cagctcgctg  | ccaatcacat  | agctttctat | ttgttaagta  | taaatttaaa | 600  |
| tttaaaatca | cttttttaac  | gaatgggggg  | aagggatcta | tgagaaaggt  | ggatcttaat | 660  |
| ttttttatag | accataaagg  | tttaaaagaa  | aataggggca | caggctgttg  | aggtttttat | 720  |
| gttgttatag | acctttttaa  | attatgttag  | agatgtatat | aggtatttaa  | aggtcactgg | 780  |
| gagcgtttct | gattcccgcc  | cacactttgc  | atttcaacac | tcagccccga  | aagatgctcg | 840  |
| ttcggtttgt | ggacctcttt  | cactccctgc  | gtgtaagaag | gtgaatcacg  | tgggaaaaag | 900  |
| tggcttttca | gtaaaacggg  | acagctcatt  | ctttctgaga | aggccccagg  | tcctctctcc | 960  |
| tcctcggatt | tgattgtctt  | ccgtgctttg  | cttcactcgt | agtaaatgac  | catccataga | 1020 |
| atatgtgaa  | ctttgtgtgag | cttcagtgagg | cagagtgaa  | tcocgcatga  | gcatttaggt | 1080 |
| gcctcgagct | gtttctgcca  | atagattaga  | aagcagccat | gagtgacag   | tccttagggc | 1140 |
| ccctgccagt | gtgcaattag  | tcattgacaa  | gaacaatgcc | atttgagagt  | gaggtgtgct | 1200 |
| ctgctgctac | gaggccattg  | tactgttttt  | tccttgaggt | caaaagcagt  | cttcccatag | 1260 |
| agtttctg   | ctctctctgt  | gacaggaaga  | aaacttcatt | accgaatcag  | agccttggtg | 1320 |
| gccactgact | ctcgtcttca  | ttgcagatgc  | tgtggttggc | ctcacaagca  | acgccttatg | 1380 |
| ctgagtgtca | gaggtgcag   | tgccactttg  | ccaaactctg | catttcattt  | catetaagge | 1440 |
| ttaacccctc | ttccttctgt  | gtgtacctgt  | gtctcctcgg | aaggaaagtc  | tagtttagat | 1500 |
| gaaacacctt | tttgtacaa   | gtaaagatca  | tctgagcaag | atgagcattt  | tgtaaaaaat | 1560 |
| aaaatgtgac | tacataaaaa  | tcaggaactt  | gacacagtgt | tgcatataata | acttttaggt | 1620 |
| cgacagcatg | tgtgtgaatc  | tcacaatcgc  | tcgtagatgt | cgctgttgg   | aaggagcag  | 1680 |
| gaggaaaggc | tgataactga  | aaatcagtag  | agtgagtgta | tccttagcaa  | cgctccagga | 1740 |
| cacttcctgt | gtgctgcag   | ttgtcagggg  | ccatttgggg | tcgccaatct  | cattctctaa | 1800 |
| aactgcttct | ttgaaacatg  | ttacttctct  | agtataatca | atgtatactc  | ctttctgggc | 1860 |
| ctgaaacgtt | gtatagctac  | ttattcagat  | actgaagacc | aacggactga  | aaaaaagacc | 1920 |
| aaacattagc | tatttttagc  | tgcaagaacc  | aggacacaca | attcgccaat  | catcccacca | 1980 |
| tataaccttc | gatttgcttt  | ctcaactcca  | ccccataatt | tctccagag   | accacttctc | 2040 |
| accttttccc | caaaagaaga  | acaaaaccag  | ttgcactcta | acactggat   | attttttctc | 2100 |
| caggggcttt | aaatagtttc  | ctatgcaacg  | tgtctgttag | cacaaaataa  | attctacaaa | 2160 |
| agttgcagta | aattttattt  | ggatatttta  | acctgttaag | tgtgtgtgtg  | ttttctgtac | 2220 |
| ccaaccagac | tttaaaataa  | acaaacatga  | aacctaaaaa | aaraaaaaaa  | aaaaaaaac  | 2280 |
| tcgag      |             |             |            |             |            | 2285 |

<210> 13  
 <211> 1805  
 <212> DNA  
 <213> Homo sapiens

<400> 13  
 agctgatgtg cactctaggt tagtaacct ttttgtgaaa aatttagaga aattctttga 60  
 gcagcttcca ctgaacaact aaaacccaat agggccaaag gccmaaacct gaggaacct 120  
 tattttattgc ttaattccaac ataggctatg aaagttttga gtttctctct gtgtattaga 180  
 atttctattcc tatttgtgtg agagagtata gtacggggaa tcagtaaat aatgaagta 240  
 aactaaagat tacacctttt ctgctggcac taagcgaaaa gccaaaccag tggctgtcta 300  
 ttatctctcg tgttttagct agtcaagtg tgagcacttg ctcttatcc tttattctct 360  
 aggggaagca agcacttat tggtcacttg gtgtccatgg ggaagaat cctaagctc 420  
 cttatgtgtt agaggacttt gttgagaatg tgaagtcgga aacatttcca gctgttaaga 480  
 tggagctgct cactgctttg ctgcgcttt tctctccc acctgctgag tgccaggaca 540  
 tgcagggacg tttgtgttat tactgcatag gtgggttttt cagaaggaaa tagtatttgc 600  
 catgaccocat agtaaaaatt cttaatagct ttcattgttt ggtgagtcac gtctgggacc 660  
 cgagaagaaa atgtgaaatg ttccataggt ttcctctctc ctccattttt ttctctatt 720  
 atggtttgca caagagtagg aggaagtggt tttgttttt aatgaagtca atctgtttgc 780  
 ttacctcaga ggaagaaaaa gatatggctg tacgggaccg aggtctcttc tattatcgcc 840  
 tctctttagt tggcattgat gaagttaagc ggattctgtg tagccctaaa tctgacctca 900  
 ctcttgagct tttggaggat ccggcagaaa gacctgtgaa tagctgggcc tcagacttca 960  
 acacactggg gccagtgatg ggcaaaagccc actgggcaac tatctctaaa tgccaggggg 1020  
 cagagcgttg tgaccagag cttcctaaaa ctctacctt tgccgcatca ggtaaaaaca 1080  
 gctcttacct taaactcttg catgataaat cttaacctt tcaatgattg gtggaagta 1140  
 gagtatctta cgaactaaac tcagactgtt gcttgaatt gaagcaagaa 1200  
 aagaggttgc tcaactggtta ttccagccat ttgtccaaaa ataaaagtca ctatgtgtac 1260  
 atttgggaat ttggataaaa aacttagctc ctacagatta agaacatctt tcacacaagt 1320  
 ttctgtcttc ttttaggacc ctggtatctc gaagagaaca aggagagggg acaagaaact 1380  
 cctgattgca gaccctcat gctagtcccc aatcgccagc ttactgctga ttattttgag 1440  
 aaaaacttgc tttagcctaa agttgctcat cagcaagtg tgctttggcg gggagaattc 1500  
 catctcgaca cccctccatg ggtctctcaa gtagtgaaca tccagacctc gccattgagt 1560  
 agggctgggt ctccggccat gaaagcctac ctacgtgctc aggatgatac gttgtgctg 1620  
 ttcttaacag aactgctatt ggagcctgga aactcagaaa tcgacatctc tgtgaacaa 1680  
 aatgaagcaa gaacggagag cgtgaatagt tttattctg tattagaaac tgtgattgga 1740  
 acaatgtgag aaataaaatc ataacagagt cttaaaaaaa aaaaaaaaaa aaaaaaaaaa 1800  
 tcgag 1805

<210> 14  
 <211> 1533  
 <212> DNA  
 <213> Homo sapiens

<400> 14  
 cctgtctgga tgactttctg cggtctgtct accctctccc ctcccgcgt cgcctgtctg 60  
 tgtcgtcggg aggtgggtga ggtgacgcga acagccccgt tgttgcctc cgcgtatccc 120  
 ctccaccct tgcggccat ccacgaacttt cgcacctctc gccattttcc gctcgttgag 180  
 ggtggacaga tgcgctcgg gtctcgccct cctgagtgcc ggtgactcgc ggaaggcagc 240  
 gatgctcttg ggggtgtgag ctggggaaat tctgtgtcac ggaatcggtg ggggtgtctg 300  
 ctcaagtctg aacggsgaga aagatgaatg ggagggtcga ttttcgagag ccgaatcgag 360  
 aggttccagc accaattccc cacatagggc ctgattacat tccaacagag gaagaaggaa 420  
 gagtctctgc agaatgcaat gatgaagct tctgggtcag atctgtgctt ttggtgcaa 480  
 caagatgatt gattactcaa ggattaatta gtaaggaaat actttcaagt catcccaaat 540  
 atggttccat ccttaaaact atacttgctt gtatcatggg atactttgct ggaacacttt 600  
 cttatgtgaa aacttgccta gagaaaatca agaaaactga aaattccccc ctggggaag 660  
 ctttcagcat aggcacaaga cgacgatctt caccacctgg gcaattat caaaagctca 720  
 aaatgagctc aagtgtgagt ggtcaatcat cttttgtgac atccccagca cgagacaaca 780  
 tagaagtctg tctctattat gagccaatc cattcaagtt tctatgata gctatctgct 840  
 ccaactggtat tactgcatcat attgtccaag gacctgatcc caaccttga gaagctcta 900  
 aaagaaaaaa tattacatat gaggaattaa ggaataagaa cagagagta taatgaagt 960  
 ctttaacaca aaagactgac cctcagctca ggcctatgca tgaagagtg ccaaaaaag 1020  
 aagtcaaaag aaacaagtat ggagatactt gggatgagtg aaaaattaca tcaattggaca 1080

|             |             |            |            |            |            |      |
|-------------|-------------|------------|------------|------------|------------|------|
| tgaaggaggtt | tcaacatcca  | gcttcactca | gggtgtcatg | attacctgca | tgctttgagc | 1140 |
| tcagcagcag  | tcttcataaa  | cacatttaaa | acaagatcct | gggtttttgt | ggtttgacct | 1200 |
| ctatggtgtt  | tttaaaaaac  | acagattttt | agtgttaata | ttgtgttaaa | gtactcacct | 1260 |
| tagggattca  | tttgaatgat  | ggtattatca | catgatgtta | tacagtttgt | gaattgtgtt | 1320 |
| caaggcaca   | gataactctt  | aaaaaacctg | cgagattaca | atgctctaga | atcagcatat | 1380 |
| aagaaaaata  | atgatattctg | catgttgaat | tggggtggat | ggggggagca | agcataattt | 1440 |
| ttaagtgtga  | agctttgcat  | caagaaatta | ttaaaaagct | ttttttctcc | aaaaaaaaaa | 1500 |
| aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa | aaa        |            |            | 1533 |

<210> 15

<211> 1758

<212> DNA

<213> Homo sapiens

<400> 15

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| gcaagtactt  | atgtggattg  | gaaaccatac  | tgaatttctg  | gatgagaaga  | ttcagccaat  | 60   |
| attagacaaa  | gtaggctctt  | tggtaaacgc  | aaggetttaa  | ttttctcggg  | gccttatgat  | 120  |
| gctgggtctt  | gagaagttag  | ccactgatat  | tcttctgtct  | ctatatgatg  | acaattctctt | 180  |
| ctgtcattgt  | gtggatgaag  | tactcttgtt  | tgaaagggag  | ctacacagtg  | ttcatggcta  | 240  |
| tctgggactt  | tttgctagtt  | gtatgcatat  | tctatcagag  | gaaacctgtt  | ttcagagatg  | 300  |
| gttgacgggt  | gagagaaaat  | ttgctctcca  | aaaaatggag  | tcaatgtctt  | cctcagaagc  | 360  |
| tgcttgggta  | tcgcaatata  | aggatatcac  | tgacgtggat  | gaaatgaaag  | ttccagattg  | 420  |
| tgacagaaat  | tttatgactc  | tactcttggt  | tataactgac  | aggtataaaa  | atcttcccac  | 480  |
| agcttcccg   | aagcttcaat  | tcttggagtt  | acagaaggac  | ttagtagatg  | attttaggat  | 540  |
| acgattaaca  | caagtgtatg  | aagaagagac  | tagagcttcc  | cttggtcttc  | gatactgtgc  | 600  |
| aatcttcaat  | gctgtgaact  | acatctcaac  | agtactagca  | gattgggctg  | acaatgtttt  | 660  |
| ctttctacaa  | cttcaacagg  | ctgcactgga  | ggtgtttgca  | gagaataata  | ctctgagtaa  | 720  |
| attgcageta  | ggacagctag  | cctctatgga  | gagctctgtc  | tttgatgaca  | tgattaaact  | 780  |
| cttagaacgt  | ttaaaagctg  | atatgttgac  | ccgtcaagta  | gaccacgttt  | ttagagaagt  | 840  |
| taaaagtctt  | gcgaataatt  | ataaaaaaga  | aagatggttg  | tcttggccat  | ctcagtcaga  | 900  |
| gcaggcagtg  | atgtccctgt  | ccagttcggc  | ttggccgttg  | ctgctgacgt  | tacgagacca  | 960  |
| tttactctag  | ttggagcagc  | agctttgttt  | ctccttattt  | aaaattttct  | ggcaaatgtct | 1020 |
| tgtagagaa   | ctggatgtat  | acatctacca  | agaaataatt  | cttgctaatc  | acttcaatga  | 1080 |
| aggaggagca  | gccacagctc  | agtttgatat  | gactcggaat  | cttttccctt  | tggtttctca  | 1140 |
| ctattgcaag  | agaccagaaa  | attattttta  | acataataaa  | gaagcctgta  | ttgttttgaa  | 1200 |
| tttgaaagct  | ggttctgcac  | tactgtctgaa | agatgtactg  | cagtcagctt  | agggcagctg  | 1260 |
| tctgtccaca  | gcagcatata  | atgaagttgg  | aatttcaaaa  | ctggctcaac  | aagatgttga  | 1320 |
| gattctactt  | aattttgagga | caaatgtggc  | taataactgga | aaaataatgtc | tttcagaaaa  | 1380 |
| aggtttctct  | ggttttttgt  | tctaagaaag  | aggaagccaa  | ttggatttca  | agttatatga  | 1440 |
| tgaattctgt  | aattaatgaa  | actggaataac | tttatagaat  | tacttattat  | cttggatttta | 1500 |
| tggtgttatt  | aaaatgctga  | ccatatttcc  | ttcatctctc  | tgttctctaa  | gaacacaaaa  | 1560 |
| cagaaaaagca | aacaatgaaa  | actcaattct  | atttacaagt  | ataaatgctg  | agtatgtctg  | 1620 |
| ttgaagacga  | gcagagatat  | taaatattaa  | ccaactttca  | atttctctgt  | ctaatttaagg | 1680 |
| gaaattctgt  | tggtgataat  | caaacatagc  | caataaattt  | ttttaaaact  | ccaaaaaaaa  | 1740 |
| aaaaaaaaaa  | aaaaaaaaaa  |             |             |             |             | 1758 |

<210> 16

<211> 1901

<212> DNA

<213> Homo sapiens

<400> 16

|            |            |            |            |             |            |     |
|------------|------------|------------|------------|-------------|------------|-----|
| gggtgccacc | taagggtcta | ggcctcttgc | tggtgcacat | gacatttgtc  | ctgcagagct | 60  |
| gggggggaag | cgatgtgtgt | accccccccc | caccgcttaa | ttttaaagctg | tttctaaaaa | 120 |
| gttgagtttc | ttctaaagag | gaagccttgc | ccagcaagga | ccagtgaagc  | agccggaatc | 180 |
| tgagacaaat | tacaagacgg | ggagtgaagc | tcttgattgc | tctggaagcc  | tgctgatttc | 240 |

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| tacaactgat  | catttgcagc  | tgctggtttt  | ggtttccacc  | ttaccctact  | ggctgtaaaa  | 300  |
| acacaaatgt  | gtactttatt  | gatttttctt  | ctaattctcc  | cgcattgggtg | gcttggggact | 360  |
| tgggagaggg  | agcaagcctt  | cctccatggc  | ccatcactcg  | gctgtggaga  | acaaagacca  | 420  |
| atgtgaagac  | actacagagg  | attctgtctt  | ccaggcccag  | tccactggggg | agtgtctggaa | 480  |
| tggggacctg  | gggtggggag  | gcagaggggtc | actttacata  | ggattaaagtt | cgagggtggct | 540  |
| accgattttca | gcacatgcac  | tactgaaatt  | tacacaaaaa  | gaaagctcag  | aaattggaagt | 600  |
| cccaattttaa | gagtcctgag  | gcagaaacctg | gtggctggag  | gcatttccag  | agggtgggaa  | 660  |
| gagagcctgc  | ccggccggag  | aacatctgcc  | ttgctgcacc  | tgaggcccag  | cagagccggt  | 720  |
| cctgggagctg | tcagataaat  | gggtgcagcg  | tgggaaggagc | ctcgcgctgc  | tggcacacagc | 780  |
| ttcacacagc  | acctctctct  | tgctgggttt  | ccacacagcc  | tgtcttcaga  | tctctgctgcc | 840  |
| gcgtgcgacc  | agagggtggga | ggccccctggt | ggcatggaa   | aggggagggtc | agtgccaagt  | 900  |
| ctcaggagga  | ggggcgcatgt | gtgtatcacc  | ctcagctggc  | ggaaacctggc | tgcgaactgt  | 960  |
| gcagttacgt  | tgcatccaca  | ggattccagt  | tggtgtctct  | tttctctctc  | tttctccgta  | 1020 |
| tttatttttt  | tattctctgg  | aggaggtgga  | catttccgaa  | gtgggtggga  | ctaagggaag  | 1080 |
| aaactctctag | ttccctcagt  | gtgaagcctg  | tcgtgtctct  | ttcccttgca  | ctggtcatca  | 1140 |
| gtatttgtta  | aaggaacaac  | tgatatactt  | gagtggtcaa  | gcaaagaacc  | catttgcact  | 1200 |
| gctgctatga  | agactacttt  | tagatcaaca  | ataaaaaaaa  | acctacaaaa  | aaacctttat  | 1260 |
| tcttttaattg | ttgcttttac  | gggtgatatt  | tgcatgcgaa  | ccaggagcat  | tttgtgtctt  | 1320 |
| aagaaaaata  | atcttagaac  | agatggctgt  | gaaaattaca  | cccatgcaca  | gaacacgcca  | 1380 |
| cagggaataat | agtcagagat  | ttggtttttc  | tctttttctt  | gtaaacctgg  | aggggtgata  | 1440 |
| tattctcttc  | atgcagttat  | tagaacttag  | ttttgttcca  | acagttaaac  | tgtcaactgt  | 1500 |
| aagaaaaatgt | gccatttttt  | tcactcagaa  | ttattcatag  | ctgtatatat  | gaaactgcta  | 1560 |
| attacacagc  | tgtgtatgtg  | gttggttttt  | tagtgcattt  | tttctctgct  | ctcatctttg  | 1620 |
| accaaactgt  | gggtatgttt  | aatatttaatt | tatatttgtc  | tcattttgta  | tgtatgtgta  | 1680 |
| gtgtgtgtgt  | gagtatgtgt  | ggtttataat  | ctgacaaaagt | catgaaagct  | agtttggctg  | 1740 |
| taattttaatt | ccctctctct  | tatttttatt  | tatttttgta  | ctgtgctgat  | tcaataaaat  | 1800 |
| gcactgacca  | tccaaaaaac  | aaacacacaa  | aaaacacaaa  | cagaaatact  | atatgtctac  | 1860 |
| atttttaaatt | cagaattagt  | atatatactt  | acacacatac  | t           |             | 1901 |

&lt;210&gt; 17

&lt;211&gt; 3145

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 17

|            |             |             |            |             |             |      |
|------------|-------------|-------------|------------|-------------|-------------|------|
| aagtgaagg  | cgaggttgct  | gtaagcggt   | aatcataggc | tgtggggaca  | agcaatgtta  | 60   |
| aaaatgaaga | aaaactaaga  | cgcagctctc  | caaacctgtc | ccgaacatct  | atacacaaag  | 120  |
| ttgactcagt | gaaaagcagc  | agaaagtact  | caaatttcca | agtgcacaaac | ggagggaatac | 180  |
| ctcgtatgca | acctcaggct  | tcagccatcc  | ctctccagg  | caaatccgt   | tcctctgcag  | 240  |
| caccattctc | tttggtctct  | cggaaccag   | tgaagcatt  | tagtaaccat  | ggctctggtt  | 300  |
| ctcctggtag | ccaagaaatt  | acacagctca  | cacaaaccac | ctctycacct  | gggctcctca  | 360  |
| tggttcagag | ccagctctca  | gcaaatctct  | ccagcaatat | caacagcgtc  | actctaacca  | 420  |
| gacctgcagg | gacaactgca  | atgagaagtg  | gcttgccacc | accagtgccc  | ccttctgctg  | 480  |
| ggggcatacc | agtgycctcg  | agcaaaactg  | ccagcctctg | ctgcagatcc  | ttgccagctc  | 540  |
| ctaaaaccta | tggtagcatc  | aaagatgaca  | gttggaagaa | tggtctttac  | tgaccagcaa  | 600  |
| agacaagaat | gcagaagctc  | acggcttcat  | ggataccctt | caccaggcta  | aaaaaacact  | 660  |
| tttatatgca | gactgttctc  | ataagactct  | tgggatttat | aaaatcccag  | ccctctctgt  | 720  |
| cttaattagc | acaaaaccag  | agagatcacc  | aaacagcact | ttaatgacat  | gttaactcag  | 780  |
| atgtgttttg | taatcatata  | atcaactctc  | atagaatcac | ttttagtctt  | gtttaataga  | 840  |
| aactaggttg | atttttaaaa  | aatatttgac  | agaggccaat | atctggggcaa | atacctaagt  | 900  |
| gatgcgcaag | agaaatgccc  | atlttgtaatt | gagaaaagt  | cccatgttgt  | acaaagaaaa  | 960  |
| tggtgaattc | aggctatcca  | aaacttcaca  | ttcaacctaa | tttactgtat  | aaatagtatc  | 1020 |
| aataaaatt  | tgtgttaagt  | agaactaata  | ttctgactaa | ttatctaaag  | gtgttcaact  | 1080 |
| gtacaccagg | aaactacaga  | ttgagattag  | gggtggggag | gaaagaaacc  | tggctgtgag  | 1140 |
| attaaaaact | tctaaaattt  | agcagaattt  | cagaaaatgt | ttttgcagaa  | tcattagaata | 1200 |
| agaaaaattg | tcattttaatc | ttaagttttg  | gatgtagctc | acatgtcacc  | accaccagat  | 1260 |
| agtgttacag | catgtatcca  | tcatgttgca  | ttgacacatc | aaacttgtgt  | gtgtttgttt  | 1320 |

|            |             |            |             |             |             |      |
|------------|-------------|------------|-------------|-------------|-------------|------|
| tgattgccaa | aagggtcttaa | tatcagttgt | acaatctttc  | tgaactttat  | agttctctggc | 1380 |
| tcaggaaaga | tggcctttgc  | tattgaagcc | aactctctca  | catgctgtta  | tctttacaga  | 1440 |
| actaagagat | ctgttttcta  | ttagcaggtt | ttcatgatat  | gaaagaaacaa | gtagtggtgtg | 1500 |
| tctttattct | tatacaaca   | ccacctcggt | gcttgcaacc  | tggaaacaaa  | ccataccatg  | 1560 |
| agakagaggg | ggaaaaaaat  | ctatgcactt | aacctacaaa  | atctctgggt  | atgacagttg  | 1620 |
| tattgttgct | attacatggc  | ataacggtct | attatgtggg  | agggaaatat  | agcctgctaa  | 1680 |
| atcctactta | agttgatcca  | ctttaaactg | agtaactgta  | taaaaactct  | attgaaaatt  | 1740 |
| cttttctctt | tgaactagat  | tctgccttac | atcaattttt  | gcatttttgg  | taaaaaaaaa  | 1800 |
| acctacttac | gtatgactct  | aacctgatac | ttgctctcta  | atggctctta  | atataatcct  | 1860 |
| gaaatcggt  | atttcaattt  | tatcagactt | ttaccagagt  | aaaacttgct  | tctgtagcag  | 1920 |
| gcctctcatt | ttttattatg  | aggctctgtt | ttaaataact  | aatttgaaca  | gctctaagat  | 1980 |
| attgtcactt | aggctcatca  | aaagctttta | gagatttgaa  | cataagttca  | tttctctgta  | 2040 |
| atcaaaagca | ttccgttaagt | tggcaaaaga | aattggggaga | gagaaataga  | aggcttgata  | 2100 |
| ttctggcagc | cattaaaggt  | gataggttga | tgataaaaaac | ttaaaaccag  | gacctccatt  | 2160 |
| ctgtcatgac | tgacaccatg  | gtagtctgtc | agcttgacca  | gtggagagtc  | attcatttag  | 2220 |
| cacaagcagc | tggagatttta | aactgccagt | actatgtatt  | tgggtgataa  | tgcaaggaag  | 2280 |
| aaactttatc | cttgaatttg  | aggggtgatg | gggtgggtcag | gaaaggatgg  | cgccagaatt  | 2340 |
| ctacatgata | atgaactaaa  | aaatgttgct | tttcagagga  | agataaagca  | tcttcttttg  | 2400 |
| ggaggggggt | atctcatgtc  | taagtaagta | aaagaaagaa  | gtagctactg  | tctcttttaa  | 2460 |
| aaaccacgta | caaaacagaa  | caagtctcag | ttttcagctg  | aacatttcaa  | aaaatatata  | 2520 |
| tgtctgaatc | taataattaa  | aagggaattt | acctattatg  | aaacwtatta  | cattttttaa  | 2580 |
| gttagataat | cagtttcaaa  | aggagtattc | aggttattta  | actttgtttt  | taaatggctg  | 2640 |
| catcagaaaa | aaatgtctat  | ttttttttat | taaaatatatt | catcacttgt  | taaaacatat  | 2700 |
| ttttgatctg | agttttggtaa | agtattattt | tacctgctgt  | tgtactacca  | cagactgttg  | 2760 |
| acttttagtt | tcttaaaagag | aaaaattgcc | tttttactag  | aaagcctttg  | tatatgtcaa  | 2820 |
| ttttcttgct | tgggaaaatc  | taaggattta | ctgtggttag  | tcttacagaa  | gaaattgtgga | 2880 |
| tttgataaac | tagtgccctat | gattttaact | tatgtttgat  | atatagtagt  | aagggtttta  | 2940 |
| tgaatgttga | ttattttgtg  | ccaacagccc | agaattgtca  | cttatatgta  | agcagaaaaac | 3000 |
| aatgagctct | gcttccaaag  | ttatttaatt | ttctcagttg  | ttgaaatgta  | ttttttgtaa  | 3060 |
| gtgtgttaat | aaaagtgtaa  | agaattggaa | aaaatatata  | tattctttaa  | tcaagcaaaa  | 3120 |
| aaaaaaaaaa | aaaaaaaaag  | cgcc       |             |             |             | 3145 |

&lt;210&gt; 18

&lt;211&gt; 2968

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (454)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1437)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (2961)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (2964)

&lt;223&gt; n equals a,t,g, or c



9

[illegible]

```
<210> 19
<211> 3953
<212> DNA
<213> Homo sapiens
```

|             |             |             |             |
|-------------|-------------|-------------|-------------|
| <400> 19    |             |             |             |
| gcggacgcgt  | ttggcaacag  | cgagggcaaa  | gagctgggtg  |
| aaacagggtc  | tggtaccctag | cttggaagat  | ttgctgttct  |
| gagaaaaatc  | ctgttcataa  | atttattaca  | gcactcaaat  |
| gatcccaagg  | tgaaaagatg  | tatggataag  | tttaagattaa |
| ggtgtcatgc  | tagacaaaag  | tctttttaaa  | aaatgtgttc  |
| acacaaagcat | ttagaagaaa  | gtttgtgatt  | ctgacttta   |
| gatgagttat  | atgaaaagtc  | tataaaagcag | ctggaggaa   |
| caactggcca  | aattcagttcc | cgattttgtg  | gggtgtgtctg |
| aggcatttcta | ctggagatac  | caaaagttccc | ttctgtcttc  |
| aaatattgcc  | ttgctgttaa  | tgactctgga  | actgaatatg  |
| gagccgagtg  | gactaaagatt | caacaaacta  | tttttgaaatg |
| ccatagtgtaa | atgtctggagc | aattgtttgtg | acttcactaa  |
| gctgaaaaat  | ttgactatgt  | catgcagttt  | ttgaaataaga |
| ggatttcagta | atgcaacggt  | tcagtctgaa  | agagaagaatg |
| ggatatttact | taaaagaaaa  | gaagtgtttt  | ccagaaggca  |
| gactttctact | tccagctgtg  | ctccattgaa  | gtgactgtg   |
| gcgacactgg  | ctaatgtgtg  | tttctgccc   | attactgggt  |
| gcagttcgaa  | atcacattgag | tttgatgcatt | ctctgtggca  |
| tttctgttcc  | atgttgtgtct | tctcgcaaaa  | cttggagttg  |
| gtcccaatg   | ttaatgggtc  | gatgtgctgg  | tctcctcttc  |
| gttaagggaa  | ttcacttttt  | tcacgatctt  | gtttctctgt  |
| aaatttgagac | actcttgcaaa | aaaacttgat  | ctctgaagag  |
| tctcttgga   | cattggcata  | tgaaaagctc  | caacaagaac  |
| tggaaaaaag  | tgtaactctga | gtcaaatgag  | gacatctcta  |
| gaaagctctg  | gagagaaaaag | ctaaaagaa   | gggttctagt  |
| aactcttcaa  | acactctttag | cttttttttg  | caagtataaa  |
| ttgtttctcaa | tcttgggtgc  | tggaagcata  | aaagcttttt  |
| aagcgagtag  | attaagaagt  | gcattttgtg  | gtcttttaaaa |
| ttgtgtttat  | tttttaaaat  | aaatataaat  | tttttttgat  |
| gttaactatg  | tggtcagata  | atccccattt  | acaatagtaa  |
| tttcaaaatt  | ccaaaattaa  | actcataagt  | tttgttgcat  |
| tttaaaacta  | atgtgatgtc  | tttagtgcca  | atagaaggta  |
| ctaaaaagag  | aaaaataatga | accccaaat   | attttattta  |
| attagcttgt  | gtttacattt  | atgccaattt  | tggtgataga  |
| atttatttca  | aaatttatca  | aatgctttta  | aatatgactc  |
| ctctctctgt  | ctgtcagagt  | tgttatatatc | aggagtgtct  |
| gccaaaagat  | ttcagatagt  | agataatgat  | gtttcatcaa  |
| ttactcgaaa  | ggagaaagtc  | tgagtgtctt  | caagtgaatt  |
| tttctatata  | ttccacatac  | ttttttctatg | ttatatattaa |
| tttttagctt  | ttacatttta  | ttactttaat  | tttataaatg  |
| agttatagca  | ttctataaag  | tttgttctat  | ttgaagtttt  |
| atttagtctg  | caggtagttaa | gtatgctact  | aaaaatcgtt  |
| ggataaaaaa  | tgcaatatgt  | agaatcaaaa  | ctgtttttta  |
| caacctgatt  | tcaagtagtt  | attcatagta  | tttatagttg  |
| aattctgtatg | gacctattca  | cactttcttc  | ttcttccata  |
| tagttccccc  | ataattttca  | gtttgttgaa  | aactgttaat  |
| aaaaacttgg  | ggtttcttag  | catgatgggt  | tatgtatgtg  |
| gtaaattatg  | tgtagcaaaa  | aagattttac  | tgagttattt  |
| atagaaaaatc | ttgatcataa  | tgctttaagt  | ttgggaactg  |
| cccttctaga  | ggtgtctggc  | aaaaagcctt  | ttgggctaac  |
| tttaaaataa  | tatatatttaa | gttgtagaggt | attttcccaa  |
| ttgttcttga  | atgttcagat  | gcatatctca  | actggtatgt  |
| tttgtgttgc  | atatttatgt  | tagttgcacc  | agggccattt  |
| ccctaatgtg  | aaaaaaggca  | ttgtgggttc  | ccctatgatc  |
| tttcatctcg  | aagtttgggt  | tacttttata  | aatgaagatt  |
| tagctgtata  | gatttcaaaa  | ttaggtttgt  | aatttgtgtc  |
| taatgtctta  | aatgcatact  | taaaaatgaa  | gtactgttat  |
|             |             |             | ctaaagctact |
|             |             |             | gtgtttagaa  |

|             |             |            |             |             |             |      |
|-------------|-------------|------------|-------------|-------------|-------------|------|
| aattgttaaga | atgagcagaa  | atttttatag | aaaagtataa  | acggaagaag  | agataagata  | 3480 |
| ctgcgaatag  | gccctcacaac | ttaaaaaaga | aaaaactttg  | ccaggttttaa | ggacatatatt | 3540 |
| tgattcttttc | agtattcttta | acaccttttt | aaacaaagtt  | cttgatagta  | cccactatta  | 3600 |
| ttgggtttgt  | tttatgccat  | tattgattct | tgatattcaa  | gcatttacaa  | tgtagcatat  | 3660 |
| ttagtttttc  | ttttcttttc  | tttttttggc | atcattaaac  | tttcatttga  | aatgcattatt | 3720 |
| gttcttgaag  | tactttgttt  | ttagcataaa | tgttgtgcat  | tttatcttag  | tgtttggatg  | 3780 |
| aaaacatttg  | tgttgttttg  | ctttcatttg | ctttgtatat  | ttaataatgt  | acctttattt  | 3840 |
| tcacgtatgc  | tacacattttg | tattgcacaa | ttaagtttatt | ttaagctgaa  | acaaaaaaaa  | 3900 |
| aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa | aaaaaaaaaa  | aaaggcgccg  | cgc         | 3953 |

&lt;210&gt; 20

&lt;211&gt; 1963

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (3)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 20

|             |             |             |            |             |             |      |
|-------------|-------------|-------------|------------|-------------|-------------|------|
| atncaagctc  | taatacgaact | cactataggg  | gggggagcgc | aagcgaggca  | gccatgtctt  | 60   |
| atcccgctga  | tgattatgag  | tctgaggcgg  | cttatgaccc | ctacgcttat  | cccagcgaat  | 120  |
| atgatatgca  | cacaggagat  | ccaaagcagg  | accttgctta | tgaacgtcag  | tatgaacagc  | 180  |
| aaacctatca  | ggtgatccct  | gagggtgatc  | aaaacttcat | ccagatattc  | cacaaaactg  | 240  |
| tctcagattt  | gattgaccag  | aaagtgtatg  | agctacaggg | cagtcgtgtc  | tccagtgatg  | 300  |
| tcattgacca  | gaaggtgtat  | gagatccagg  | acatctatga | gaacagctgg  | accaagctga  | 360  |
| ctgaagaagt  | cttccaagaat | acaccttggc  | ccgaggctga | agccatttgt  | ccacagggtg  | 420  |
| gcaatgatgc  | tgtcttctct  | attttataca  | aagaattata | ctacaggcac  | atatatgccca | 480  |
| aagtcagtgg  | gggaccttcc  | ttggagcaga  | ggtttgaatc | ctattacaac  | tactgcaatc  | 540  |
| tcttcaacta  | cattctttaa  | gccgatggtc  | ctgctccctc | tgaactaccc  | aacagctggc  | 600  |
| tctgggata   | tatcgatgag  | ttcatctacc  | agtttcagtc | attcagtcag  | taccgctgta  | 660  |
| agactgccaa  | gaagtcagag  | gaggagattg  | actttctctg | ttccaatccc  | aaaacttgga  | 720  |
| atgttctaat  | tgtctctaat  | gtccttctatt | ccctggtaga | caaatccaac  | atcaaacccg  | 780  |
| agttggagggt | atacacaaag  | ggaggtgacc  | ctgaagatgt | ggctggggag  | tatggccggc  | 840  |
| actccctcta  | caaaaatgctt | ggttacttca  | gcctggctcg | gcttctccgc  | ctgcactccc  | 900  |
| tgttaggaga  | ttactaccag  | gccatcaagg  | tgctggagaa | catcgaaactg | aacaagaaga  | 960  |
| gtatgtattc  | ccgtgtgccca | gagtgccagg  | tcaccacata | ctattatgtt  | gggtttgtcat | 1020 |
| atttgatgat  | gcgtcgcttac | caggatgccca | tcggggctct | cgccaacatc  | ctcctctaca  | 1080 |
| tcacaggagc  | caagagcatg  | ttccagagga  | ccacgtacaa | gatgatgagt  | atttaacaagc | 1140 |
| agaatgagca  | gatgcctgcy  | ctgctggcca  | ttgccctcac | gatgtacccc  | atgcgtatgy  | 1200 |
| atgagagcat  | tcacctctcac | ctgcggggaga | aatatgggga | caagatgctg  | cccatgcaga  | 1260 |
| aaggtagccc  | acaagtctcat | gaagaacttt  | tcagttactc | ctgcccacaag | ttcctgtcgc  | 1320 |
| ctgtagtgc   | caactatgat  | aatgtgcacc  | ccaactacca | caaagagccc  | ttcctgcagc  | 1380 |
| agctgaaggt  | gtttctctcat | gaagtacagc  | agcaggccca | gctttcaacc  | atccgcagact | 1440 |
| tccctgaagct | ctacaccacc  | atgcctgtgg  | ccaagctggc | tggtctctctg | gactctcagc  | 1500 |
| agcaggaggt  | ccggatccag  | cttcttgyct  | tcaaacacaa | gatgaagaac  | ctcgtgtgga  | 1560 |
| ccagcggtat  | ctcagccctc  | gatgggtgaat | ttcagtcagc | ctcagaggtt  | gactctcaca  | 1620 |
| ttgatgaagg  | cttatgccat  | atcgcggaca  | ccaaggtcgc | caggcgctgt  | ggcatttctt  | 1680 |
| tcactccgca  | gatccacaaa  | tttgaggagc  | ttaatcgaa  | cctgaagaag  | atgggacaga  | 1740 |
| gaccttgatg  | atattcacac  | acattcagga  | acctgttttg | atgtattata  | ggcaggaagt  | 1800 |
| gtttttgtga  | ccgtgaaaac  | tttacctaga  | tcagccatca | gcctgtcaac  | tcagtttaaca | 1860 |
| agttaaagtc  | cgaaagtgtt  | caagtggatc  | tcagtaaaag | atctttggag  | ccagaaaaaa  | 1920 |
| aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa | aaa         |             | 1963 |

&lt;210&gt; 21

<211> 3045  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (128)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (141)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (739)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (770)  
 <223> n equals a,t,g, or c

<400> 21  
 cccagaattg tccctccaaa gcccccaccc cataaaagcc attgtcctcc tccctcgacc 60  
 cctctgtgat ccttggttag agagcctttt ccaactgtgag gaagtgtgga aaaatagcct 120  
 ctgtctgngt gtgtgtgtgt ntgtgtgtgt gtgtgtaatc tgttaggttg gggatagggt 180  
 ttctctctagc caatattaaa agagacctgc aataaaaaaa ttacctctgat ctgatagaaa 240  
 gcaagtgttt ttgtatgtgt ggggtgaatgt gtgttcatgc ccgtatatgt ctacacacag 300  
 atgacaaatt atatttgaaa tcgttggaaa ataaattcag atcaaaaatgc ctttcaggcc 360  
 cattacctag aaatctatct taaaacctgg gtatgttcc t aaggctcatt ctttgccttat 420  
 gctaaaattaa ttacaattat gaatggagga tattctactg tactttttta aaaagaaact 480  
 atttttgtgt ttgaaagtga aaccaacatc cagatctata gcagagtcct tattctctc 540  
 ataaatcttt ttactttggc tacaataaga tgatggatg attctattat atatttwata 600  
 taaaatccat ccaaattaag ttttgggtaa gtgtgttgtt taatctgaac tatagtaact 660  
 taatactcta aacaatagtt cactccattt ggctcttct ccacagatgt aattatgttt 720  
 tcaactcagg aactatggnc aaggaacttt cccagatca aattcyatn aacgctgaga 780  
 tacaagtcac ccattgcacag ccactatcat acccttttat ctactcgaaa ggcagaactc 840  
 agaacctgtt attttatgtc tgtaaatcat tactttggca tcttttgtag gaaaggggca 900  
 ggataactca ctggaatgta cagtattttg ctagtgcatt tcaaggaatg gaactcttct 960  
 cagttaacaac ccacaccgtc ttctctcagg gatttccaac tggcactctg tgggtgctac 1020  
 acagaatgca atttaagtga tatttctcag cctgggtcag aataaattga tctcttgatc 1080  
 ccagaagata tatactgaag tgtgggataa agattatgat tagggggagg ttggagacaa 1140  
 aagctgtaaa ttactatggc tgattttatt ctactatata catatatatt ttttgccttt 1200  
 gtatattcta tataggaaac taagcattgt atttttttta acaaatctaa aaaagcacta 1260  
 tgaactacag gtgtttgaat ttcaaaaatc attttgtatt gttaatatct tcaacttgtg 1320  
 tgaactctgg aagctgcaga tctttgtctg gacgcaataa atttatatac tttttgaggg 1380  
 gttctctctg ggtgtcaatc aggcacctgt tatgtctagg gggagccctg gtgctacttg 1440  
 cttgaaagtt tcagtgtaaq taccctgatg ccttttggac cttgggagca gatcaagagt 1500  
 ttggagatc aggtaccaag gaaataagga cagtctagct gcctcaagtg agggggccct 1560  
 tgcatagtct tccctcccc tcaactgaagc tgggtagcct attggggttg agagggaaaa 1620  
 tgtgaaattc cagaatttat ctcccttaga agagagccag taacttatgt acaaggatga 1680  
 aagaaggctc gcagcagtag ctttggggaa agggaggaa atatggcact tctccaaccc 1740  
 cggaaaaatc tgcctttgaa aactgctgat aaaaattag ccggttatta ctctcgtttg 1800  
 ggagactgtg ctctctgtgt tgcctctctt ggctctactc cacagatacc agactctctc 1860  
 taagaggatg agcagaccag ctttgaggtt gacctgtttc tctttgtctg ccttcccaaa 1920  
 acaccagccc ccaggaagac attaaagcag ctttaagctta aattctact cctctctcca 1980

|             |            |            |            |            |             |      |
|-------------|------------|------------|------------|------------|-------------|------|
| aatttggctc  | acttgcccta | gatccaagcg | agggaaagga | aaagaagggg | ggctctctggc | 2040 |
| tttattactc  | ccctaagctc | ttactctgac | ttccccaaac | ccagaagaag | ttctctccaca | 2100 |
| gtgtttcatt  | gaaagaggag | tattttgtcc | cattttcccc | ttcctcatta | tcaaacagcc  | 2160 |
| ccagttcttc  | ttgtctctgc | taagaaagta | gaggcatgat | gatctgcctc | tcaactgcc   | 2220 |
| taagtctctag | ctaagtatca | ggggaaaaaa | aaaaaaaaaa | agcctaacaa | attgggattag | 2280 |
| actaggcgctg | caaagtatga | ggattttgtt | gatccctctg | ctgggatgtg | tgtcttccca  | 2340 |
| tatcttgccct | tcaggaatta | cactgtgcct | tttccccagg | gatatgggct | ctgtctaccc  | 2400 |
| agtgtcccgag | tttccccgta | actgtctctg | aacattgttg | acaagggcag | gtcttcatat  | 2460 |
| ttttgatcat  | ccctttctcc | cagtgaatac | ccatagccct | tacctagagt | ctagggcaca  | 2520 |
| aagacttcgg  | ggaagataca | ctgagattga | cctgaggaga | catctacaca | caccagtggc  | 2580 |
| agctgcccca  | gggctctgct | cccccttcta | agtctgtcat | cctctggaag | ggatgggtgg  | 2640 |
| tgtccaatc   | tctgggtgct | aaaaacccaa | gtttatttct | ctcttaacac | tggcaataac  | 2700 |
| cagtccaac   | cactgttgcc | ttttaaaacc | tcttaataat | ctcatgtgtg | gtttgttttg  | 2760 |
| attccaatcc  | aattatcacc | aggcctgtgt | gggtaaatgc | ttttaaatgc | tctctcatct  | 2820 |
| tgktcttccc  | cctcaccccc | cactcttagg | tatgtatgat | gctaactctg | tccttaagta  | 2880 |
| agtttcttcc  | tgtctctttt | gtatcttctt | ttctgtctct | tcctcctacc | ttttgtctct  | 2940 |
| tgggtgtttg  | ggaacttttt | tttttttttt | ggccttttgt | acaaagatta | gtttcaatgt  | 3000 |
| agctctgtag  | ctcctttgta | aaccaattaa | aaagtttttt | aataa      |             | 3045 |

<210> 22  
 <211> 446  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (12)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (26)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (441)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (446)  
 <223> n equals a,t,g, or c

|            |            |            |            |             |            |     |
|------------|------------|------------|------------|-------------|------------|-----|
| <400> 22   |            |            |            |             |            |     |
| gctgtttccc | angctaaagt | tccgtngcac | aatcacggct | cactgcaccc  | tcaacctcat | 60  |
| ccttccaagt | agctcagact | ataagcacac | gccccgttac | tttgatgctc  | aaagtctccc | 120 |
| aggtgtggcc | atgggagggc | tcagtggctt | ctctgttgcc | tagggccatt  | gggttggggt | 180 |
| gctctcaggt | ttctgtttgt | agatactctg | tgtctgtctt | gtatgtcttc  | aacctgggaa | 240 |
| gcagccattt | ctccaaggag | ccctgtgtcc | ctttagtga  | gggtgggtctg | gatcttgggt | 300 |
| agaagccaa  | atcaagattc | tgggtgtgct | cacagctcta | gggtgttagc  | agacagagct | 360 |
| ggaagtgtgt | tatgtgaata | caagtgcaca | gttgtgggtg | cgtctgtgta  | tgtgcgaata | 420 |
| catgcgtctt | tgtatatgat | naaaan     |            |             |            | 446 |

<210> 23  
 <211> 3397

<212> DNA  
 <213> Homo sapiens  
 <220>  
 <221> SITE  
 <222> (1)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (15)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (24)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (3081)  
 <223> n equals a,t,g, or c

<400> 23  
 nggattcgcg gcegnctccga ctgnccgcgg ggctagcact gacgtgtctc tcggcggagc 60  
 tgctgtgocag tggaaacgcgc tgggccgcgg gcagcgtgcg ctcacgcgga gcagagctga 120  
 gctgaagcgg gaccgcggagc cagagcagcc gccgccatgg caatcaaatt tctggaagtc 180  
 atcaagccct tctgtgtcat cctgccgga attcagaagc cagagaggaa gattcagttt 240  
 aaggagaaa tgctgtggac cgctatcacc ctctttatct tcttagtggt ctgccagatt 300  
 cccctgtttg ggaatcatgt ttcagattca gctgaccctt totattggat gagagtgtatt 360  
 ctagcctcta acagaggcac attgatggag ctagggatct ctctattgt cactgttggc 420  
 cttataatgc aactcttggc tggcgccaag ataattgaag ttggtgacac cccaaaagac 480  
 cgagctctct tcaacggagc ccaaaagtta ttggcatga toattactat cggccagctct 540  
 atcgtgtatg tgatgaccgg gatgtatggg gacccttctg aaatgggtgc tgggaatttg 600  
 ctgctaatca ccattcagct ctgtgttgcg ggcttaattg tctactttt ggaatgaact 660  
 ctgcataaag gatattggct tggctctggt atttctctct toattgcaac taacatctgt 720  
 gaaaccatcg tatggaagcg attcagcccc actactgtca acactggcgc aggaatggaa 780  
 tttgaaggtg ctatcatcgc acttttccat ctgctggcca cagcgacaga caaggtccga 840  
 gcccttcggg aggcgttcta ccgcagaaat ctcccaact tcatgaatct catcgccacc 900  
 atctttgtct ttgcagtggt catctatttc cagggcttcc gagtgacct gccaatcaag 960  
 tcggcccgct acctgtggca gtacaacacc tatccatca agctcttcta taactccaac 1020  
 atccccatca tctctcagtc tgccttggtg tccaaacctt atgtcatctc ccaaatgctc 1080  
 toagctcgct tcaagtgcga ctgtctggtc agcctgtggt gcacctggct ggacacgtct 1140  
 tctggggggc cagcacgtgc ttatccagtc ggtggccttt gctattacct gteccctcca 1200  
 tggctcatga actcaacggg tacatcccca cagccgcggc ctltgggtgg ctgtgcacgt 1260  
 gggccctctc ggtctctggt gaactcctag gcgcatttgg gtctggaacc gggatcctgc 1320  
 tgcagtcgac aatcatctac cagtaactttg agatcttgt taaggagaca agcgaggttg 1380  
 gcagcatggg ggcctctctc ttctgagccc gtctcccgga caggttgagg aagctgtctc 1440  
 agaagcgctc cggaaagggga gctctcatca tggcgcgctg tgetcgggca tatggacttt 1500  
 taataatggt ttgaaatttc gtattctttc attccactgt gtaaaagtct agacatttcc 1560  
 caatttaaaa ttttgcattt tatcctggca ctggcaaaaa gaaactgtga agtgaatttt 1620  
 tattcagcgc actgccagaa aagtgggaat ggtataggat tgtccccaa gtcctcatga 1680  
 acttttgttt taacctttgc accttctcag tctgtatgc ggcgcagcc gtctcactgt 1740  
 ttccccaca aagggaattt ctcaactctg ttggaagcac aaacactgaa atgtctacgt 1800  
 ttcatttggg cagtagggtt tgaagctggg agcagatcat gtatttcccc gagacgtggg 1860  
 accttctgtg catgtctctc tcaacaatcag cgttgggaat atctggctta ggaactgttc 1920  
 tctctaaagc acctattggt tcccttattt taaaagtgat ttttttaagg acagaacttc 1980  
 ttccaaaaga gagggatggc ttccccagaa gacactcctg gccatctgtg gatttgtctg 2040

|            |             |             |             |             |             |      |
|------------|-------------|-------------|-------------|-------------|-------------|------|
| tgcacctatt | ggctcttcta  | gctgactctt  | ctgggtgggc  | ttagagtcgt  | ctgtttcttg  | 2100 |
| ctagctccgt | gttttagtcca | cttgggtcat  | cagctctgcc  | aagctgagcc  | tggccaagct  | 2160 |
| aggtggacag | acccttgacg  | tgatgtccgt  | ttgtccagat  | tctgccagtc  | atcactggac  | 2220 |
| acgtctcttc | gcagctgccc  | tagcaagggg  | agacattgtg  | gtagctatca  | gacatggaca  | 2280 |
| gaaactgact | tagtgctcac  | aagcccttac  | accttctggg  | ctgaagatca  | cccagctgtg  | 2340 |
| ttcagaattt | tcttactgtg  | cttaggactg  | cagccaagt   | agcagacacc  | accgacttcc  | 2400 |
| ttttctgcgt | accagtgctc  | tcagcagaga  | gaggacagca  | caggctcaag  | gttggtagt   | 2460 |
| aagtccaggt | cggggtgcat  | gggctgtggt  | ggltgtgatc  | agttgtctcca | gtgttctgaa  | 2520 |
| taagaagact | catgtttatg  | tctggaataa  | gttctgtttg  | tgctgacagg  | tggcctaggt  | 2580 |
| cctggagatg | agcaccctct  | ctctggcctt  | tagggagctc  | cctcttagga  | caggcactgc  | 2640 |
| ccagcagcaa | gggcagcaga  | gttgggtgct  | aagatcctga  | ggagctcgag  | gtttcgagct  | 2700 |
| ggctttagac | atttggtgga  | ccaaggatgt  | tttgacaggat | gccctgaccc  | taagaagggg  | 2760 |
| gcctgggggt | gcgtgcagcc  | tgctggggag  | accycactgc  | tgrcagtgct  | agccaggaaa  | 2820 |
| cagagtgcac | aagggacaa   | aagggacttg  | cctaaagcca  | cccagcaact  | cagcagcaga  | 2880 |
| accaagatgg | gcccccaggct | cctccatagt  | gcccagggct  | taccacccta  | tcacacgtgg  | 2940 |
| cctgtgctag | accagtcct   | gagcaggggga | gaggctcttg  | agacctgatg  | ccctctacc   | 3000 |
| cacatgggtc | teccactgcc  | ctgtctgtct  | tgctgtctaca | gagggggcagg | gcctccccca  | 3060 |
| gcccacgctt | aggaatgctt  | ngcctctggc  | aggcaggcag  | ctgtaccctaa | gctggtggggc | 3120 |
| agggggcttg | aaggcaccag  | gcctcaggag  | gagcccccata | gtcccgcctg  | cagcctgttaa | 3180 |
| ccatcggtgt | gccctgcaag  | gcccacactc  | acgcctgtgt  | ggtgatgttc  | acgggtgggtg | 3240 |
| gggtgggggt | gaccccaact  | tccaggggac  | tgctactgtg  | gacgcacaaa  | ttggcataact | 3300 |
| gagataaggt | gaataagtga  | caataaagc   | cagtttttta  | caaggtaaaa  | aaaaaaaaaa  | 3360 |
| aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaa     |             |             | 3397 |

<210> 24  
 <211> 2836  
 <212> DNA  
 <213> Homo sapiens  
 <220>  
 <221> SITE  
 <222> (1615)  
 <223> n equals a,t,g, or c

|             |             |            |            |             |             |      |
|-------------|-------------|------------|------------|-------------|-------------|------|
| <400> 24    |             |            |            |             |             |      |
| gcgcctcgcc  | gggtctccgc  | ttatcacacc | tcagggtcgg | tgggcttcgg  | gtgggggggc  | 60   |
| tcagctagc   | tgatggcaag  | ggaggaaatg | cagggtgggg | gatttggtgt  | tgccagaggt  | 120  |
| cccgcgacg   | gggggctcgg  | gggtctcttc | agacgagatt | cccttcaggc  | ttggggcaga  | 180  |
| gtccctctgc  | acggagatcc  | caatgaacgc | gggccccttg | aggccggttg  | ttggggcttc  | 240  |
| tcgcgctcgc  | ggatggggcc  | gttaccctar | ccgctttcca | gcgcctcagt  | cggttcccca  | 300  |
| tgccctcaga  | ggatggccgg  | ggaagcgcgc | cgccctcttc | ttcgtctcgt  | tggccactgt  | 360  |
| gctggggcta  | cgctctgggt  | ggaagaccac | ggagacctac | cgggcctcgt  | tgctcttactc | 420  |
| ccagatcagt  | ggcctgaatg  | cccttcagct | ccgctcatgt | gtgcctgtca  | ctgtcgtgtt  | 480  |
| tacgcgggag  | tcagtgccct  | tggacgacca | ggagaagctg | cccttcaccg  | ttgtgcattga | 540  |
| aagagagatt  | cctctgaatt  | acaaaatgaa | aatcaaatgc | cgttttccaga | aggcctatcg  | 600  |
| cagggctttg  | gacctatggg  | aggaggccct | gtcatcgggc | agtggtgcaag | aggcagaagc  | 660  |
| gagtttagat  | gagcctcagg  | acaaagcggg | gggtccctg  | actgtgtacg  | gtatatctga  | 720  |
| acactcctca  | ctcttctccc  | aggacatgat | gagctacatt | gggcccacaga | ggacagcagt  | 780  |
| gggtgggggg  | ataatgcacc  | gggaggccct | taacatcatt | ggccgcgcga  | tagtccaggt  | 840  |
| ggcccaggcc  | atgtctttga  | ctgaggatgt | gcttctgtct | gctctggctg  | accaccttcc  | 900  |
| agaggacaaq  | tggagcgtct  | agaagaggcg | gcctctcaag | tcagctttgg  | gctatgagat  | 960  |
| caaccttcagt | ttactcaacc  | cagaccccaa | gtcccatgat | gtctactggg  | acattgaggg  | 1020 |
| gggtgtctgt  | cgctatggcg  | aacctttctc | gaatgccctc | ggtgcgctgt  | gcacctcttc  | 1080 |
| tgttgactct  | cgatattcttt | actatgcaat | gttgggggtg | aatccccgct  | ttgactcagc  | 1140 |
| ttctccagc   | tactatttgg  | acatgcacag | cctcccctac | gtcatcaca   | gtgtggagtc  | 1200 |
| ccggtctggga | tcagtgctgt  | cctccttgta | cctgtgtctc | aactttctac  | tctactgtgc  | 1260 |
| tgagcttgca  | cactcaccgc  | tgtacattca | ggacaaggat | ggcgctccag  | tggccaccaa  | 1320 |

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| tgccttccat  | agtcceccgt  | ggggtggcat  | tatggtatat  | aatgttgact  | ccaaaaccta  | 1380 |
| taatgcctca  | gtgctgccag  | tgagagtcga  | gggtggacatg | gtgcgagtga  | tggagggtgtt | 1440 |
| cctggccacag | ttgcgggttc  | tctttgggat  | tgctcagccc  | cagctgcctc  | caaaaatgcct | 1500 |
| gctttcaggg  | cctacagagt  | aagggtcaat  | gacctgggag  | ctagaccggc  | tgctctgggc  | 1560 |
| tcggtcagtg  | gagaacctgg  | ccacagccac  | caccaccctt  | acctccctgg  | cgcantttct  | 1620 |
| gggcaagatc  | agcaaacatt  | tcattaaagg  | cgacgtggca  | tctgaggtgt  | acaaggctgt  | 1680 |
| agctgccctc  | cagaagtcgc  | cagaagagtt  | ggcgtctggg  | cacctggcat  | ctgcctttgt  | 1740 |
| cgccagccag  | gaagcttga   | catcctctga  | gttgcccttc  | tttgaccctg  | cactcctcca  | 1800 |
| ctcctcttat  | ttccctgatg  | accagaagtt  | tgccatctac  | atccacctct  | tccctgcctat | 1860 |
| ggctgtgcc   | atccctcctg  | ccctgggtcaa | gatcttccgt  | gagaccgccca | agtcctggag  | 1920 |
| aaagcctgag  | aagacagact  | gagcaggcca  | gcacctccat  | aggaagcctt  | cctttctggc  | 1980 |
| caaggtgggc  | gggtgttagat | tgtgaggcac  | gtacatgggg  | cctgccggaa  | tgacttaaat  | 2040 |
| atttgtctcc  | agtcctccact | gttggtctctc | cagcaaccaa  | agtaacaacac | tccaagatgg  | 2100 |
| gttccatctt  | tcttctcttc  | ccattccact  | ggctcaatcc  | tctctccaca  | ccaggggcct  | 2160 |
| caaaaggcac  | atcatccggg  | tctccttctc  | ttgtttgata  | agggctgtgc  | ctgtctccct  | 2220 |
| ctgtggcaag  | gactgtttgt  | tcttttggcc  | catttctcaa  | catagcacac  | ttgtgcactg  | 2280 |
| agaggaggga  | gcattatggg  | aaagtccctg  | ccttccacac  | ctctctctag  | tccctgtggg  | 2340 |
| acagccctag  | ccctctgtgt  | catgaagggg  | ccaggcatgt  | gtcacctgtg  | ggacctctct  | 2400 |
| ctcctcctcc  | ctccctccta  | gttggctttg  | tctgtcaggt  | gcagctctgg  | gggtacccag  | 2460 |
| gaggcagag   | ctcaggcaat  | gggtgtgtgt  | gtgtgtgtgt  | gtgtgtgtgt  | gtgtgtgtgt  | 2520 |
| gtgtcagagg  | ttccagaact  | ttccagattt  | ggaatcaaac  | agtcctgaat  | ccaaatcctt  | 2580 |
| gtttttgcac  | ttattgtctg  | gagagctttg  | gataaggtat  | tgaaatctct  | tgagcctcag  | 2640 |
| tttttcaatt  | gttcaaatgg  | cactgatgat  | gtctccctta  | caagatgggt  | gtgagagatg  | 2700 |
| aatgtgatca  | gcattgtaag  | tgtctggcgt  | gtagtaggct  | cttaataaac  | actggctgaa  | 2760 |
| tatgaattgg  | aatgawaaaa  | aaaaaaaaaa  | aatttcggaa  | ttcgatatca  | agctttatcga | 2820 |
| taccgtcgac  | ctccga      |             |             |             |             | 2836 |

&lt;210&gt; 25

&lt;211&gt; 20101

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 25

|             |            |            |            |            |             |      |
|-------------|------------|------------|------------|------------|-------------|------|
| agccggcctc  | gcacttccgg | tggggagatt | ccggcctgga | gctcccaggg | ccgagcagac  | 60   |
| ctttgggacct | gtgagcgtg  | catccaatta | accatgggaa | gggtcagcac | cagccaccag  | 120  |
| ccctctagggt | gaggactctg | cctggggctc | tgctgatggg | tccgaatcat | ggagctgcag  | 180  |
| agagctctct  | cagcctggag | acgttcttgg | tgaaaagctg | ggcttaacct | ccacggctct  | 240  |
| tgtctcaaat  | tgatccaag  | aggggtgect | gcccccgtg  | actcaggagc | tccgggtgctg | 300  |
| cagccgccac  | gaatggggag | gtgggcccct | gatgtggcct | ttttgtggaa | ggcgggtgtg  | 360  |
| acctctgggc  | tggtgctttc | ctactactgc | ttctccatcg | gcataccact | ctacacaaag  | 420  |
| tggtgacaaa  | agagcttcca | tttccccctc | ttcatgacga | tgctgcacct | ggccgtgatc  | 480  |
| ttctctctct  | cgccctctgc | cagggcgctg | gttcagtgct | ccagccacag | ggcccctgtg  | 540  |
| gtgctgagct  | gggcgcacta | cctcagaaga | gtggctccca | cagctctggc | gacggcgctt  | 600  |
| cagctgggct  | tgctccaact | gagcttccgt | tatgtcaccc | tctcgctgta | cacaatgacc  | 660  |
| aaatccctgac | ctgtctctct | catcttgatc | ttctctctga | tcttcaagct | ggaggagcgtg | 720  |
| cgccggccac  | tggtcctcgt | gggtccctct | atccggggg  | gtctcttcat | gttcaactac  | 780  |
| aagtccacac  | agttcaactg | ggagggtctc | gcttgggtgt | ggggggcctg | ttcatcggtg  | 840  |
| gcattctgct  | gacctctacc | cagatgtctc | tgcagaaggc | tgaactcggc | ctccagaatc  | 900  |
| ccatcgacac  | catgttccac | ctgcagccac | tcatgttctc | ggggctcttc | ctctcttttg  | 960  |
| ctgtatttga  | aggtctccat | ttgtccacat | ctgagaaaaa | cttccgtttc | caggacacag  | 1020 |
| ggctctctct  | tgctgtaact | gggagcctct | tctttggcgg | gattctcgcc | tttgggtttg  | 1080 |
| gcttctcaga  | gttctctcgt | gtctccagaa | cctccagcct | cactctctcc | attgcccggca | 1140 |
| tttttaagg   | agctctgact | ttgctgttgg | cagctcaact | gtggggcgat | cagatcagcc  | 1200 |
| tctgtaactg  | ctggggctgc | gcctctgctc | ctcgggaaat | tctctccagc | ttgccctcaa  | 1260 |
| agccctgcat  | tccagargtg | atggtggccc | caaggccttg | aaggggctgg | gctccagccc  | 1320 |
| cgacctggag  | ctgctctccc | ggagcagcca | gcgggaggaa | ggtagacaat | aggaggagga  | 1380 |
| gtactttgtg  | gcccaggggc | agcagtgacc | agccagggca | aatggcttag | aagcaggcca  | 1440 |



|            |             |             |            |             |             |      |
|------------|-------------|-------------|------------|-------------|-------------|------|
| ctccccagcc | tgtctgccagc | actcaactgtg | ctcaagccgc | cagggtccat  | catggtagct  | 1500 |
| gggagctgtg | gacgggagtc  | accaggtggt  | ggggccaagc | cagggaactca | tgaacttttgc | 1560 |
| ccctcccttc | agagcctgg   | cacacaagg   | gcgagccca  | ggccagcctg  | ggactggcca  | 1620 |
| gagctggggc | caagctcgcc  | tggaaatgca  | gcaggagagg | ggagtgggct  | ggttctctccc | 1680 |
| accactcccc | aggctctgac  | agccgagact  | catttccaag | gcacagcagc  | tttctaaagg  | 1740 |
| gactgagttt | ggactggggt  | ttggacctcc  | aggggctgga | gcttcatcac  | ctgggagctg  | 1800 |
| tcttttttca | gagagcaggt  | tcttttatag  | tttggaaata | aatgggtcac  | ggtccactgg  | 1860 |
| ccgcttctgt | tgtctggaga  | ctgtgggggca | gggaggggac | agtgtgggcc  | tggcctctcc  | 1920 |
| ttctctttcc | ctgcctggag  | ccttcttcaa  | atgtctggtc | ttaagccagg  | cctctctcat  | 1980 |
| ttctctgctc | ctgttagaac  | accagtcctc  | tycccaagtg | ggccccactg  | caactgctgt  | 2040 |
| caggaaataa | atgaatgttt  | actgagwaaa  | aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | 2100 |
| a          |             |             |            |             |             | 2101 |

&lt;210&gt; 26

&lt;211&gt; 2046

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 26

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| gtcatgcagt  | gcgcgggaga  | actgtgctct  | ttagggccga  | cgctaggggc  | ccggaaggga  | 60   |
| aactgcgagg  | cgaagggtgac | cggggaaccga | gcatttcaga  | tctgctcggt  | agacctgggtg | 120  |
| caccaccacc  | atgttggctg  | caaggctgggt | gtgctctcgg  | acactacctt  | ctagggttttt | 180  |
| ccaccagcgt  | ttcaccagg   | cctccctctg  | tgtgaagaat  | tccatcacga  | agaatcaatg  | 240  |
| gctgttaaca  | cctagcagg   | aatatgccac  | caaaacaaga  | attgggattcc | ggcgtgggag  | 300  |
| aactggccaa  | gaactcaaa   | aggcagcatt  | ggaaccatcg  | atggaaaaaa  | tatttaaaat  | 360  |
| tgatcagatg  | ggaagatggt  | ttgttctggt  | aggggctgct  | gttggttctg  | gagcaattgtg | 420  |
| ctactatggc  | tgggactgt   | ctaattgagat | tggagctatt  | gaaaaggctg  | taatttgggc  | 480  |
| tcagtatgtc  | aaggatagaa  | ttcattccac  | ctatatgtac  | ttagcaggga  | gtatttgggtt | 540  |
| aaagcctttg  | tctgccatag  | caatcagcag  | aacgcctggt  | ctcatgaact  | tcagtgtgag  | 600  |
| aggctctttg  | gtgacaattg  | gtgtgacctt  | tgcagccatg  | gttggagctg  | gaatgtctgt  | 660  |
| acgatcaata  | ccatatgacc  | agagccagg   | cccaaagcat  | cttgccttgg  | gtctacattc  | 720  |
| tgtgtgtgat  | ggcgagctgg  | tggctcctct  | gacaaatatta | gggggtctct  | ttctcatcag  | 780  |
| agctgcagtg  | tacacagctg  | gcattgtggg  | aggcctctcc  | actgtggcca  | tgtgtgcgcc  | 840  |
| cagtgaaaaa  | tttctgaaca  | tgggtgcacc  | cctgggagtg  | ggcctgggtc  | tctgtcttgt  | 900  |
| gtcctcattg  | ggatctatgt  | ttcttccacc  | taccaaccgtg | gctggtgccca | ctctttactc  | 960  |
| agtggccaatg | tacgggtggat | tagttctttt  | cagcatgttc  | cttctgtatg  | ataccagaaa  | 1020 |
| agtaatacag  | cgtgcgagaag | tatcaccaat  | gtatggagtt  | caaaaatag   | ataccattaa  | 1080 |
| ctcgatgtgtg | agtatctaca  | tggatatact  | aaatatattt  | atccgagttg  | ctcatttgtct | 1140 |
| ggcaactgga  | ggcaacagaa  | agaaatgaag  | tgaactcagct | tctggctctt  | ctgtcatcatc | 1200 |
| aaatatcttg  | tttaatgggg  | cagatatgca  | ttaaatagtt  | tgtacaagca  | gctttcggtg  | 1260 |
| aagtttagaa  | gataaagaac  | atgtcatcat  | attttaaagt  | tcgggttaatg | tgatgctcca  | 1320 |
| ggcttgcctt  | tttttctgga  | gaataaatgc  | agtaactctc  | ttccaaataa  | gcacacacat  | 1380 |
| tttcaattct  | catgttttag  | tgaattttaa  | atgttttggg  | gaatgtgaaa  | actaaagttt  | 1440 |
| gtgtcatgag  | aatgtaagtc  | ttttttctac  | ttttaaattt  | agttaggttga | ataactttta  | 1500 |
| aaaatttagc  | aaacctgtgt  | tgtcatattt  | ttttggagtg  | cagaatattg  | taattaatgt  | 1560 |
| catagtgtat  | tggagcttgt  | ggtaaaaggga | ccagagagaa  | ggagtcacct  | gagctctttt  | 1620 |
| gtttttttaa  | atacttagaa  | cttagcactt  | gtgttattga  | ttagttagga  | gccagtaaga  | 1680 |
| aaactctggg  | tatttggaaa  | caagtggtca  | tgtttacatt  | catctgtcgtg | acttaacaaa  | 1740 |
| actgttcact  | ctgaaacagg  | ccagaggtgat | gcatttctct  | gctgttctgt  | ctcagtgctc  | 1800 |
| tctttccaat  | atagatgtgg  | tcatgtttga  | cttgtacaga  | atgttaatca  | tacagagaat  | 1860 |
| ctttgatgga  | attatatatt  | tgtgttttac  | ttttgaatgt  | tacaaaaggga | aataacttta  | 1920 |
| aaactattct  | caagagaaaa  | tattcaaaagc | atgaaatatt  | tgtctttttc  | cagaatacaa  | 1980 |
| acagtatact  | catgagcaaa  | aaaaaaaaaa  | ggcgcgccgc  | tctagaggat  | ccctcgaggg  | 2040 |
| gcccaa      |             |             |             |             |             | 2046 |

&lt;210&gt; 27

&lt;211&gt; 3569

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (903)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 27

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| gcccacgcgt  | cggggggatt  | cgtcgcggcg  | ccttctgagt  | ggtcggggtcg | aggcttctctg | 60   |
| gcctagcagtg | gcctctcgctg | cgcgatctca  | ggcgggttct  | cctcggtctcc | gcgcagcccg  | 120  |
| cgcccgcggtg | ggggaccgcg  | cgcagcggcg  | cctgctgcg   | agggaccgccg | cgcccgccccc | 180  |
| cggtgctcgt  | gatggggctg  | atcttcgcca  | aactgtggag  | cctcttctgt  | aaccaagaac  | 240  |
| acaaagtaat  | tatagtggga  | ctggataatg  | cagggaaaac  | caccattctt  | taccaattctt | 300  |
| taatgaatga  | agtggttcat  | acttctccaa  | ccataggaag  | caatgttgaa  | gaaatagttg  | 360  |
| tgaagaacac  | tcattttctt  | atgtgggata  | ttggtgggtca | ggagtctctg  | cgatcatcct  | 420  |
| ggaacacata  | ttaactcaaa  | acagagtcca  | tcattcttgt  | tgttgatagc  | attgacaggg  | 480  |
| aacgactagc  | tattacaaaa  | gaagaattat  | acagaatggt  | ggctcatgag  | gattttacgga | 540  |
| aggctgcagtg | ccttatcttt  | gcaaaataac  | aggatatgaa  | aggggtgatg  | acagcagctg  | 600  |
| aaatctcgaa  | ataacctcacc | cttagttcaa  | ttaaggatca  | tccatggcac  | attcaactct  | 660  |
| gctgtgctct  | cacaggagaa  | gggttatgcc  | aagggtctaga | gtggatgacc  | tcccggaattg | 720  |
| gtgtgagata  | acttttttgc  | tgtgaaagaga | gtgctctatt  | tattctgtga  | catgaacatt  | 780  |
| tttcctagtg  | acctttgggt  | gctaaggcag  | cagcatgttt  | aatttataac  | aacacaaaac  | 840  |
| ttctgacagca | acaactgaat  | caagtgcacg  | tgaactggaa  | cataaaaagt  | tttttcttaa  | 900  |
| ttnttttttt  | tttaacacac  | taactttcag  | ttggatgaat  | gtaatgtata  | actatgtttt  | 960  |
| cagcaacaat  | tctttctctg  | attctaatta  | atcagtgact  | gccttgttaag | ccattgtttgt | 1020 |
| catatgcgtg  | atgtcttctg  | aaatattctt  | ataaccttaa  | tgaccaattg  | ctttcaattc  | 1080 |
| ttgacacagca | ctccctccca  | accagagaat  | tactgggttg  | atagatagtg  | cttggaaaac  | 1140 |
| atcaggtact  | gcctcgacag  | ctctccagca  | ctaaatatat  | ttgtctccct  | tataaacgga  | 1200 |
| tcactctttg  | gacagaaact  | actgtaaaag  | aagaaatctg  | cctagaggat  | atatgtaaag  | 1260 |
| aagattccac  | atcatgagta  | cttgctcttt  | aactttcccc  | cacattactg  | ttggatcatg  | 1320 |
| gaataatggt  | taagtgtgta  | tttgcatgga  | aattaagtag  | gctgtttatt  | tatctaaagg  | 1380 |
| aatcaagtc   | actcttctgc  | ctgcaacatt  | tgttcaaaaa  | ctaaccaagg  | taaaaatttt  | 1440 |
| atttgaagc   | ccaactttga  | tgttaaatat  | tcttgaataa  | atctgttatt  | ttaagaatat  | 1500 |
| cacattattc  | aatgcataata | aaactatcag  | aagttagtaa  | atcataccag  | cactaaaaat  | 1560 |
| aagacaattg  | gaatatattt  | tagcatcagt  | ttacaaacaa  | ctttattatc  | aacagaaaat  | 1620 |
| ttagctcttt  | tctttgcaag  | atatatcaca  | gctgctttgg  | gcagtagctg  | aagccgaagt  | 1680 |
| atgaacagtc  | cattttgktt  | cttaaaattt  | gaagtcgtgt  | ctgtcatagc  | atttttacta  | 1740 |
| ccagcagtat  | gttaactaaa  | aaactacatg  | gctttccctg  | aatttatttg  | acggwtwtat  | 1800 |
| gtaataakct  | tgaacaactg  | gccatctttg  | tagttatgoc  | ttggggttcta | taaatgktaa  | 1860 |
| ggaaactgct  | gaattgtaac  | ctagctttta  | gaatattaat  | aggagactca  | aagtttaatat | 1920 |
| tcttaacaaa  | ttaaaaattaa | aaaaagtcac  | aactctacaa  | aagtttattt  | attggatggc  | 1980 |
| attaaaaact  | ttttgagggca | gttgcttaat  | atgaacaata  | atctaaacaa  | tatatctcct  | 2040 |
| aaattaaagag | gaagacaag   | gtgctgattt  | cgatcctgkt  | tattcaaatg  | tggtgatttg  | 2100 |
| ctgaaatgaa  | agggataaaa  | tgaatactta  | gagaataata  | cttcattttt  | cgagctattt  | 2160 |
| ttaatttttg  | taaaaaaagt  | acaaagttca  | taatatcaaa  | atatgttgta  | aaaattggtg  | 2220 |
| ttttaatttt  | aatatttttg  | taagttgatt  | taaattttgc  | tctttttttc  | agggtgcttt  | 2280 |
| ggtttattat  | atttagtcaa  | gttaatttag  | togaatgtgg  | taacattctt  | ctgatttatc  | 2340 |
| ttttagttgga | gcacaccctt  | gaactgaaca  | gtggccaaag  | gaaagcttct  | ttggcttttg  | 2400 |
| aaagttagttg | tttaactttg  | ttttaagccg  | ttttcttagt  | gattctgtat  | caagttaggg  | 2460 |
| ataagtgwag  | tttaactttg  | tttttaatat  | aaccataat   | caagaacatg  | agcgaaaaag  | 2520 |
| agacataaat  | cgatatggat  | ggtttgggtg  | gtgtgtggga  | tggtggggaa  | ttaagaaaaa  | 2580 |
| gccatttacc  | taagcacagt  | ttgcctgaat  | ttctgcttgg  | ttgtgttgtt  | taccgtaagt  | 2640 |
| actgagggtga | gtttccctaa  | gtcgtctcgt  | agaaaaagca  | tacttagtag  | ctctgtattt  | 2700 |
| gttcttctga  | atagtactat  | tgccctcttg  | tatctagtga  | gattggctgg  | ctcaattttc  | 2760 |
| ttctgtcaaa  | ttatatgggt  | attttttata  | ttaccacatc  | agcattatat  | taaaagtggt  | 2820 |
| tttaatatggt | gattgtattt  | tgccaactac  | tagtatagac  | tcaaatttgc  | tattttaattt | 2880 |

|             |             |             |            |            |             |      |
|-------------|-------------|-------------|------------|------------|-------------|------|
| ttaaaatata  | atattattttg | taaatccttt  | aaaaaatatt | tggttagttt | tggattagaa  | 2940 |
| atgatttatg  | ttagccatgt  | gttgaagatg  | aaattggcat | cagtgtagac | ggtgctgatt  | 3000 |
| gggaaaagt   | catgattagg  | aaattcatgt  | aagacttttt | aagagtattt | tttaggtttt  | 3060 |
| cactcaatct  | ttcctgtcac  | agtaacgtga  | aaactgatta | ttctttactc | caagagaatg  | 3120 |
| tttttaaccca | agcaagtatc  | taataactaga | gcattggttc | tgacattcat | tgtaaatcag  | 3180 |
| tgaactgttaa | gatactgtct  | aaaaaatgta  | catttgtaat | tagtgccttt | attcatattt  | 3240 |
| tgaataaagt  | tcttagtttt  | atattatacc  | tgttctttag | aagtcagtgt | agcagctaatt | 3300 |
| tatttcagtg  | atgaaataat  | ttgtgtttta  | tatccccctt | cacctccatg | tattggttag  | 3360 |
| cacagtttat  | agtaagttaa  | aagcaaggga  | cccacttaca | ttaccttcag | gagactgttc  | 3420 |
| taccttttaa  | tttgaaaaca  | aggaaattaa  | attttaacag | aaatctttta | agtatgttat  | 3480 |
| aaccaaagt   | ttcaattatc  | cttgcaagtg  | caaatcacgc | atttttctta | aaaaaaaaa   | 3540 |
| aaaaaaaaa   | aaaaaaaaa   | ggcggccac   |            |            |             | 3569 |

&lt;210&gt; 28

&lt;211&gt; 2556

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (72)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (141)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (294)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (303)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 28

|             |             |            |             |             |             |      |
|-------------|-------------|------------|-------------|-------------|-------------|------|
| attctacagc  | agaataact   | tggaaaaact | caagtttttc  | cacatacttg  | gaaaaactct  | 60   |
| ctagcatttg  | tnngaaattta | gtgtaattaa | gtgaaacctt  | cctagcaact  | gtagtaattta | 120  |
| aaagcgtggg  | gtgtgctttt  | ngttgagatc | cgtgaatggt  | tctgtaaaaca | caattttgat  | 180  |
| tgtgttcgac  | ttcttaaaag  | ttgtgatgac | aacggttaatt | tttaaccactt | gaccgtatat  | 240  |
| cgttttcatc  | cttgaagact  | gtcatatatt | tccaagtgtc  | tttccctccct | ggttaatttta | 300  |
| ggnntaaagt  | cgaggtccgg  | aagccacctt | ggagattttt  | taattgggtt  | tattggagca  | 360  |
| tttaacctggg | agcgatcctg  | tcgttaggtg | gcattgccta  | tattcagcag  | aacgtcaagt  | 420  |
| ttgtcactgg  | ttatgcgatc  | cccactgtct | gcgtggcctt  | tgcttttgtg  | gycttccctc  | 480  |
| gtggccagag  | cgtttttcac  | accaagcttc | ctgatggcag  | tgcttccacc  | gayatgttca  | 540  |
| agatactgac  | gtattcctgc  | tgttcccaga | agcgaaatgg  | agagcgccag  | agtaatgggtg | 600  |
| aaagcattgg  | agctctttcag | caatcttcta | aacaaagtct  | gttttgattca | tgtaagatgt  | 660  |
| ctcatggttg  | gccatttcaca | tggaagatgt | gaaagctctg  | gtccaagattg |             | 720  |
| tcctctgttt  | cttggtcttg  | ataccttact | ggacagtgtt  | tttccaattg  | cagacaacat  | 780  |
| atgtttttaca | gagttctcat  | ttgaggattc | cagaaatttc  | aaatattaca  | accactcttc  | 840  |
| acacgtctcc  | tgacgtctgg  | ctgacacctg | ttgatgctgt  | gctcatctct  | ctgctcatcc  | 900  |
| ctctgaaggga | caaatctggtc | gatcccattt | tgagaagaca  | tgacctgctc  | ccatctctcc  | 960  |
| tgaagaggat  | cgccgtgggc  | atgtttcttg | tcattgtgct  | rgccttgctt  | gcagggaattt | 1020 |
| tggagagtaa  | aaggctgaac  | cttggttaag | agaaaacctt  | taatcagacc  | atcggaacag  | 1080 |

|            |             |             |             |             |             |      |
|------------|-------------|-------------|-------------|-------------|-------------|------|
| tcgtctacca | tgctgccgat  | ctgtcgtctg  | ggtggcaggt  | gccgcagtac  | ttgctgattg  | 1140 |
| ggatcagcga | gatctttgca  | agtatcgacg  | gcctggaatt  | tgcaatactca | gctgccccca  | 1200 |
| agtccatgca | gagtgccata  | atgggcttgt  | tctttttctt  | ctctggcgtc  | gggtcgttcg  | 1260 |
| tggtttctgg | actgctggca  | ctgggtctcta | tcaaaagccat | cggatggatg  | agcagtcaca  | 1320 |
| cagactttgg | taataattaac | ggctgctatt  | tgaactatta  | ctttttcctt  | ctggctgcta  | 1380 |
| ttcaaggagc | tacctctctg  | cttttctcta  | ttatttctgt  | gaaatatgac  | catcatcgag  | 1440 |
| acctacagcg | atcaagagcc  | aatggcgctgc | ccaccagcag  | gagggcctga  | ccttctcgag  | 1500 |
| gccatgtcgg | gtttctgagg  | ctgacatgtc  | agtaactgac  | tggggtgcac  | tgagaacagg  | 1560 |
| caagacttta | aattcccata  | aaatgtctga  | cttcaactgaa | acttgcatgt  | tgccctggatt | 1620 |
| gattttctct | ttccctctat  | ccaaaggagc  | ttggtaagtg  | ccttactgca  | gcgtgtctct  | 1680 |
| tgccacgctg | ggccctcccg  | gaggagagct  | gcagatttcg  | agtatgtcgc  | ttgtcattca  | 1740 |
| aggtctctgt | gaatcctcta  | gctgggttcc  | cttttttaca  | gaaactcaca  | aatgggagatt | 1800 |
| gcaagctctt | ggggaaactcc | acgtgttagt  | tggcatccca  | gtttcttaaa  | caaatagtat  | 1860 |
| cacctgcttc | ccatagccat  | atctcactgt  | aaaaaaaaaa  | attaataaac  | tggtacttat  | 1920 |
| atttaagaaa | gtgaggattt  | tttttttttt  | aaagataaaa  | gcataggctag | atgctgcaag  | 1980 |
| gattttacat | aaatgccata  | tttatggttt  | ccttctcgag  | aacaactctg  | ctcttgccat  | 2040 |
| gttctttgat | ttaggctggg  | agtaaacaca  | tttcatctgc  | tgcttcaaaa  | agtaacttact | 2100 |
| ttttaaacca | tcaacattac  | ttttctttct  | taaggcaagg  | catgcataag  | agtcatttga  | 2160 |
| gacctgtgtg | cccatctcaa  | gccacagagc  | aactcacggg  | gtacttcaca  | ccttacctag  | 2220 |
| tcagagtgtc | tatatatagc  | tttattttgg  | tacgattgag  | actaaagact  | gatcatgggt  | 2280 |
| gtatgttaag | aaaacattct  | tttgaacaga  | aatagtgtaa  | ttaaaaataa  | ttgaaagtgt  | 2340 |
| taaatgtgaa | cttgagctgt  | ttgaccagtc  | acatttttgt  | attgttactg  | tcgctgtatc  | 2400 |
| tggggctctc | ccggtttgtt  | atactttttc  | tgattttgtt  | gctgtatttt  | tgccataact  | 2460 |
| ttattataaa | aagcatctca  | aatgcgaaat  | ccaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa  | 2520 |
| aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | actcga      |             |             | 2556 |

<210> 29  
 <211> 1149  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (1138)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (1139)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (1140)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (1143)  
 <223> n equals a,t,g, or c

|            |            |
|------------|------------|
| <400> 29   |            |
| cggggcaacc | actatagggg |
| aaagctggat | gacctgaggt |
| accggctcgg | aattccccgg |
| 60         |            |
| tcgaccacac | cgctccgcgc |
| gggggtctgt | ctgagaataa |
| tgggcccggt | ggccccggac |
| 120        |            |
| gagtggaatg | attaatgatg |
| ttttgcagca | gttttctacg |
| ctgaaatttt | tttatgtctc |
| 180        |            |
| tggaaccyag | aatttgctaa |
| gagatggagg | aaacctcaga |
| aagctatgtg | aaacaaatgg |
| 240        |            |
| accttgagag | agatgaacct |
| ctcaaaagca | cgggccctca |
| gatttctgtg | gtctcgtctg |
| 300        |            |

|             |             |             |             |              |             |      |
|-------------|-------------|-------------|-------------|--------------|-------------|------|
| tctatcactg  | gagcagcagg  | gaatctgaac  | acgacctcct  | ggteccacaag  | gctgtggccca | 360  |
| aatggagcgc  | ggaagaagtt  | gtcctctggc  | tggagcagct  | gggccctctgg  | gcattctcttt | 420  |
| acagggaag   | gtttttatct  | gaacagagtaa | atggaaaggtg | aggagcaaaag  | tctttctgaca | 480  |
| caccgtggac  | atttttagaaa | acagctttct  | tgagatataaa | tccaggccag   | gcgcgggtggc | 540  |
| tcacacctgg  | atccccagcgt | tttgggaggc  | cgaggcggat  | cgatcatttg   | aggctcaggag | 600  |
| ttccagacca  | gcctgaccaa  | tatgatgraa  | ccccatctct  | actaaaaata   | caaaaaaaata | 660  |
| aaattggcca  | ggcttggtat  | tgcacacctt  | gtaatcccat  | ctattcggga   | ggctgagaca  | 720  |
| ggagaatcac  | tgaaacccaa  | gaggtggagg  | ttacaatgag  | ccaagatcac   | ggcactgcac  | 780  |
| tccagcctag  | gcaacaaggc  | aagactccat  | ctcaaaaaaga | gagagagaga   | gatgtaattct | 840  |
| acataaaatt  | cacacgtttg  | aagtgcacaa  | tccatgggttt | ttaattgtgtt  | cacaaaatagt | 900  |
| gtgatattaca | ccacagctgg  | ttttagaaca  | ttttcatcac  | cctaaaaagaa  | agctttttacc | 960  |
| cttcagtcaca | tctctatttc  | tccccaaccc  | ccagccctctg | gcacaaacacca | tctacttttc  | 1020 |
| tgtctttatg  | gattttgtcta | ttctggacgt  | ttgatataaa  | tggattcata   | caaaaaaaata | 1080 |
| aaaaaagggc  | ggccgctcta  | gaggatccaa  | gcttacgtac  | gggtgcattga  | accgaacnann | 1140 |
| tanccggac   |             |             |             |              |             | 1149 |

&lt;210&gt; 30

&lt;211&gt; 1867

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 30

|              |             |             |             |             |             |      |
|--------------|-------------|-------------|-------------|-------------|-------------|------|
| gccacgcgct   | ccggcctcag  | cctcccagct  | agctgggact  | acaggcgtga  | gcaccatgcc  | 60   |
| cgccctcaac   | gatattgatct | ctttgggctg  | tagtcagtat  | tggattatga  | tcaattattat | 120  |
| caccatttat   | tttgtgtctc  | cagttctctc  | agctgtggcc  | aatcctctacg | tgggattcttt | 180  |
| gtgcccctac   | aacattctctc | atcctggctt  | tttgttttga  | gcacttctct  | cttctcctage | 240  |
| accaccaggc   | tcttgtatta  | tccctgtccc  | tgcctcggaa  | tgcactctc   | ctccagagag  | 300  |
| ccctgtgttc   | ttttgttaga  | ggatgtgata  | tagaatccaa  | catgcagaca  | cttggtggag  | 360  |
| ttattgttat   | tggggttttg  | ttatactagg  | gtttcagtg   | tcagtgtcag  | tatttatgta  | 420  |
| tgttaaccaca  | cgctgtgctt  | tggattcagg  | ctatttcaaa  | ttttagataaa | tatggtacat  | 480  |
| atattatttaa  | taccactagt  | tactacattg  | gtacttttca  | gcacaaatata | tctaaagtggg | 540  |
| atcaaatgag   | actgttaata  | gctttacatc  | agttcagggtc | agttatgttg  | ctaaaattact | 600  |
| tttggcatta   | agtttaggga  | aaaaaaattg  | ggtttgggat  | tttttgggtt  | caacattttgt | 660  |
| gatttgagaga  | ctatggacct  | gtaataagtc  | caagaacagc  | agttgtcagtg | taacaggagt  | 720  |
| gttaattggaa  | tcgggtcatt  | tagaaacagt  | caagacttcg  | ctgttgtgca  | tgtggttagg  | 780  |
| agccagtgca   | cacgtcagtt  | cttaggaaat  | gtacagtcgt  | agcaaatagca | tttgaattcc  | 840  |
| aagactcttc   | ccatttgtgt  | gctgttgagt  | gtagaaaaata | aatgtgtgta  | atttctttat  | 900  |
| cttgagttat   | gagattctcc  | ccttagaata  | aaacaaagaa  | ttttctctca  | gtgtaaaaat  | 960  |
| gtcaagtttt   | attctctgaaa | tgaatagcaa  | agtttaagctt | aaaaacgtga  | acagcttcag  | 1020 |
| aactataaat   | gggtatgtat  | acctttctgc  | tgtctaaagg  | cagagaaagtg | aaagaaagtg  | 1080 |
| tgggtgtctt   | cagaggagac  | agcagcaaga  | cacatttgtga | cagaaaaacca | agggattctct | 1140 |
| gtgtcacagt   | gaaatgtgaa  | gagggcacct  | ctcctttcaa  | gagacgaaag  | tctgatacat  | 1200 |
| gggaagcaca   | ctctccgctg  | tgtgtgtctc  | aggagagggtg | caccctgtat  | ggaaataattt | 1260 |
| gggaagggtta  | agattaagac  | agggttaaaa  | aaagcaagg   | caaatcacaa  | agcaagggtt  | 1320 |
| aattgttaata  | tgaaaaagtc  | agaaattcaag | gaaaaagcat  | ggggacaaaag | agaatttttc  | 1380 |
| ctctttttgg   | tgtcgtctca  | tgtgttagcct | acaacagaa   | tataagagctt | atagacattt  | 1440 |
| atatgaatat   | ttatttgaaa  | acgtataata  | tcaaacaaatg | taaaagccaa  | tagaattctc  | 1500 |
| agataattga   | atgtatagaa  | actagcagtt  | tgaagtgtat  | tagtttcaata | tgttgtgatc  | 1560 |
| aagcagaaaa   | ataagcatat  | gaagatat    | taaaatggga  | tttaataagtg | tgttataaca  | 1620 |
| gatcctattc   | catgtccttt  | gaatatattat | agaaattaaa  | tggaaacaaat | tagggcatca  | 1680 |
| ggaaaagggtta | acaaaagatc  | ttaccaaaaa  | arggaaaawat | atatatatat  | gtgtagtact  | 1740 |
| acctatatata  | atacataata  | tatagtactg  | cttatatata  | tatatgccta  | tatgtcacaa  | 1800 |
| tatatatata   | catgtatagg  | cagtactatg  | ttttctgatc  | ataatatgtt  | aaattagtaa  | 1860 |
| aaaaaaa      |             |             |             |             |             | 1867 |

&lt;210&gt; 31

&lt;211&gt; 1272

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 31

|            |             |             |            |             |             |      |
|------------|-------------|-------------|------------|-------------|-------------|------|
| ccacgcgctc | cgggatgctt  | tttgccagca  | atgtgagaaa | agggtgctct  | ctggggagaga | 60   |
| ggaagagacc | caggcaattt  | attatgaatg  | ctcccgttg  | tatggataat  | ctgtgtctcc  | 120  |
| ttgcgccaat | tccgcacact  | ctggaaactt  | cattcacatt | tggccacagat | gagccactcc  | 180  |
| tcctctcttc | cccttgagaa  | tcgttctctg  | agcctgtgca | gaacaagggtg | ctccgatccct | 240  |
| ctatcttgca | cactggctcc  | tttctctccc  | ccaactgtct | ccctgtccac  | ctgtgtctgt  | 300  |
| gctctccagt | ctctctcttg  | tagtttcttc  | ctcagaagac | agtgtcgccc  | ctcactgtctc | 360  |
| ctctgaccag | gggtccatat  | ttctaacttt  | ggaagtgcct | ctctggacatg | tcctatgtggt | 420  |
| tgcttgggca | tcactcaaaa  | tcagcctct   | ccaaaaggaa | tgattctccc  | ctacttctct  | 480  |
| ctcacacaa  | tgtgtggcca  | gagtagccgg  | accaatggct | ccaaactacc  | cccaataact  | 540  |
| catccccgcc | tcamtgttg   | ggcccccttg  | gcttccccta | gggcagctca  | catcaagggtc | 600  |
| cagcttgat  | cggagctcct  | acaggaagct  | tcccccgcc  | tgctctgtcg  | gagaaactctt | 660  |
| ctctccata  | ctamctctcc  | cattctgtgg  | caggctcttc | tttaycctca  | ggcttcagyt  | 720  |
| cagacwtccc | tcacctgcta  | ggccacagca  | gctcttgagt | agetgggatt  | acaggcacc   | 780  |
| gcgcgtgcta | atttttgtat  | ttttagttag  | gatgggggtt | tcaccatatt  | ggtcaggctg  | 840  |
| gtctcgaaat | ctgacctaca  | ggtgatcaac  | ccacctgggc | ctcccttaaat | gcggggatta  | 900  |
| caggcatgag | ccacgcctcc  | cagcctttga  | ttttttaagg | tggattttgg  | ttgttataaa  | 960  |
| tggaagaagg | taagagtcca  | agttcaaccc  | gtgtgtgaaa | gcaaaacaat  | ggaaaaacagg | 1020 |
| attggtctct | tcaaaaggctc | ctctgttaga  | actgcctctt | tgaaattctg  | aggttaactca | 1080 |
| ctttggagac | ctctgctgca  | gagggctcgt  | tcctaagtta | aaagcatcgc  | taacctctgg  | 1140 |
| ctctgtgggc | atttttcaaaa | ggtttaaaagg | aattgtatcc | cttgaaaggc  | ctgaaaaata  | 1200 |
| aaaagtcttt | aacatacaaa  | aaaaaaaaaa  | aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | 1260 |
| aaaaaaactc | ga          |             |            |             |             | 1272 |

&lt;210&gt; 32

&lt;211&gt; 2235

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1097)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 32

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| cacgagggaa  | ttacaggcgt  | gaactaccgt  | gcccagcctt  | tttttcatag  | cagttttatt  | 60   |
| aagttgtatt  | tgccatacca  | cccaatgtat  | ccattttaagc | acctgatcca  | gtgggtttttc | 120  |
| atgtactcat  | ggagttaagc  | agccaacaatc | ttagcgcatt  | ttcattacc   | caaaaagaaa  | 180  |
| ctgtaccact  | tatgcacccc  | gttcccctcc  | tcgggtctcg  | gcaaccacga  | gtctactgtc  | 240  |
| tgctctcatg  | gatttgccca  | ttctcgacgt  | ttcattggga  | tgaaatcaca  | cagtgtatgg  | 300  |
| cttcccacac  | tttactgtgc  | tggtgtcaag  | gtttatctat  | gtgttggtgtg | cagccacccc  | 360  |
| ttggtatcca  | cagggatagg  | accagggagc  | ctgcaccgat  | ccctgcagg   | gatgcctgtg  | 420  |
| tcccacagtg  | cccctgcga   | aactcactga  | tatgaagagt  | cgccctctcg  | tatccatggg  | 480  |
| cttcagatcc  | cgtgattact  | gtatctcttg  | ctctgtgtga  | gttgaatctg  | cgggtgtaga  | 540  |
| accacagac   | acagggaagt  | gctgtagctt  | atccccttgt  | atggtcagag  | agtttccgt   | 600  |
| agcggggatg  | gacacgtgtt  | catttcaactt | tcactgatg   | ggcatcggga  | atgtttccac  | 660  |
| ttctgtctca  | tcatttaacaa | ttctgctaca  | aacatacatg  | tacttctgtt  | ggaggggaca  | 720  |
| catgttttca  | tttctctctg  | gtgtgtctat  | ccaggagtgg  | aattggctgg  | gtcttatggt  | 780  |
| aaactctgtta | actgtttttt  | gcttttttgt  | ttttttgttg  | ttgttttttt  | gagacggagt  | 840  |
| ttaccctctg  | ctgtgaacta  | ccataggtcg  | gagtacaagt  | gcgcaatccc  | agctcagcac  | 900  |
| aaactctgcc  | tcgtagact   | aaagccagcag | ggcagagctc  | taccctcgag  | tgaaagtggg  | 960  |
| ctttgccttc  | tcgtgccacg  | agkacttgct  | ggcaccctac  | cttgagccca  | tcagtgaggaa | 1020 |
| ataccacagc  | ccatgaggag  | tggaaatgag  | gaagtgtctg  | ctgatgtccg  | caccatcgtg  | 1080 |

|             |             |             |            |             |             |      |
|-------------|-------------|-------------|------------|-------------|-------------|------|
| aaccagatca  | gctacanccc  | ccaggatccc  | cgagacctct | gtggacgcat  | actgaccacc  | 1140 |
| tgctacatgg  | ccagcaagaa  | ctcctcccag  | gagacgtgca | cccgggcccag | agagttggcc  | 1200 |
| cagcagattg  | gaagccacca  | catcagtcctc | aacatcgatc | cagccgtgaa  | ggcgtcatg   | 1260 |
| ggcatcttca  | gcctggtgac  | ggggaagagc  | cctctgtttg | cagctcatgg  | aggaagcagc  | 1320 |
| agggaaaacc  | tggcgctgca  | aaatgtgcag  | gctcgaatac | ggatggctct  | gccttatctg  | 1380 |
| tttgctcagt  | tgaagcctctg | gtctcggggt  | gtccacgggt | ggctcctcgt  | gctgggattc  | 1440 |
| gccaaactgg  | atgagagctct | cctgggctac  | ctgaccaagt | acgactgctc  | cagtcgggac  | 1500 |
| atcaaaccca  | taggcgggat  | cagcaagacg  | gacctcaggg | ccttcgtcca  | gtctcgatc   | 1560 |
| cagcgcttcc  | agcttctctg  | cctgcagagc  | atcctgttgg | cgcggccac   | cgcagagctg  | 1620 |
| gagcccttgg  | ctgatggaca  | gggtgtcccag | accgacgagg | aagatatggg  | gatgacatat  | 1680 |
| gcggagctct  | cggtctatgg  | gaaactcagg  | aaggtggcca | agatggggcc  | ctacagcatg  | 1740 |
| ttctgcanaac | tcctcgcat   | gtggagacac  | atctgcaccc | cagacagagt  | cgtcgacaaa  | 1800 |
| gtgaagcggt  | ttttctccaa  | gtactccatg  | aacagacaca | agatgaccac  | gctcacaccc  | 1860 |
| gcgtaccacg  | ccgagaacta  | cagccctgag  | gacaacaggt | ttgatctgcg  | accattttctg | 1920 |
| tacaacacaa  | gctggccttg  | gcagtttccg  | tgcatagaaa | atcaggtgct  | acagctcgag  | 1980 |
| agggcgagagc | cacagtcctc  | ggacggcggt  | gactgaggcc | ggttccttcc  | tggaggcctc  | 2040 |
| ctgtcctcgg  | ggaccccagc  | acctcatcat  | cagcattgct | ggagccaagg  | gtaggagccc  | 2100 |
| tacactagga  | gccccaggatg | ggacggcgca  | tcagccgaga | gggaggggaa  | ttttcagttca | 2160 |
| aattcctcaa  | aaagaggctg  | gaataaagcc  | tgggcttaaa | aagaaaaaaa  | aaaaaaaaaa  | 2220 |
| aaaaaaaact  | cgtag       |             |            |             |             | 2235 |

&lt;210&gt; 33

&lt;211&gt; 1963

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (2)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (15)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1871)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1954)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 33

|             |             |             |            |             |             |     |
|-------------|-------------|-------------|------------|-------------|-------------|-----|
| gnaactctct  | atatngaatt  | ctgggtacgc  | tgcaggtagc | gggtccggaat | tcocggggtcg | 60  |
| accacagcgt  | ccggtatgaag | cccatgccgc  | tgtctgaggg | caagtctata  | ctgctgtttg  | 120 |
| gaggggctgc  | tgtctgttgc  | atcctggcag  | tggccatcgg | ggtagccctg  | gctctgagaa  | 180 |
| agaaatagga  | ggcttttcag  | aagagaaaaga | cagaaggatg | taaggtttga  | gttgatttgg  | 240 |
| ctggaatttg  | aacctccagc  | agctgtctgg  | acatttgttg | aacctcttgg  | gataaattggg | 300 |
| gaactctcgt  | caacatggca  | gtggcatgtt  | agggatgtta | gggcttgagg  | tggggcattc  | 360 |
| acattctatc  | gactgtaaat  | ccccagggcc  | tcgcctcatg | ctaaatttga  | aatcttaggg  | 420 |
| gtaaaagcacc | ccctccagga  | ccgggtttct  | cagccttggc | actagtgctg  | ttctgaccat  | 480 |
| tctctgtgtt  | ggggctgtcc  | tgtgtgtggt  | gggtccacc  | cactagatgc  | cagtgaccac  | 540 |
| ccctcccaga  | gatgacaaac  | gaaaatgtct  | ctagacattg | ccaaatgtcc  | cgtgtgaaca  | 600 |

|             |            |            |            |             |             |      |
|-------------|------------|------------|------------|-------------|-------------|------|
| tccccattg   | agacccactg | ctttagcgag | agagggttta | cttaggaaga  | attgggatag  | 660  |
| aaatccccg   | ctgagagaa  | ttagctgtgg | gctctcagc  | tactgacttc  | ttagctctta  | 720  |
| atccccattg  | aatttcacat | tctctgatga | gcaggtctgt | caccactct   | tttttgccc   | 780  |
| ccccccctca  | tctctggatg | tgaggggtgt | cgcccgta   | ctcagctg    | tctcaggagc  | 840  |
| tgcactgttc  | ctcttcaccc | ccaggttctc | gctaagatcc | cacggcgagc  | ggctgtgctt  | 900  |
| ggactcagtc  | tgtcaagatc | ccgaagcttc | ctgcagctcc | acctgtgtaa  | aatgtgctct  | 960  |
| ttgggaaatc  | tcaaaaatct | tacacagaga | aaatcacatg | aaggagacat  | ggggccccca  | 1020 |
| cttgtgagtg  | caactctcag | taactctggc | tagagagaca | tactgtgtct  | gtgtcaaggc  | 1080 |
| aggaggatga  | ccyggatgac | cttctgaggt | ctcttcagcc | cttttcgcta  | gtggtcaccc  | 1140 |
| accacatggt  | ttacttgcca | gcaacatctc | tattgtcgga | tggtccctgt  | ctataacctt  | 1200 |
| gggtcagtat  | attttttcca | atatgggacc | ttagtcttac | tactgatgag  | ttctatgggt  | 1260 |
| ctctgtctag  | ggggtaagga | tttttattct | tgggcttata | gagccagtta  | gatcataaatt | 1320 |
| cttatgaaat  | agagagtgtc | ctaaatatca | ctgaaataaa | aagttaggaaa | aagaagcttg  | 1380 |
| aattttaaga  | ctgaggtctc | tctgcagatt | ctagtgtggc | tttcagagtt  | caagagtgtg  | 1440 |
| ggcatcttca  | ctgtaattct | tcaatgccag | ggtaataaac | caaaatagtc  | ctaatcagta  | 1500 |
| tatgtcagtt  | gagcatcgcc | ataattttct | ttctctggc  | tgatcccgag  | ctaaaggaa   | 1560 |
| gggttagaccc | gtgtctttcc | agccctaaag | gaagggtaga | cccggtgtct  | tccagcccta  | 1620 |
| aaggaggggc  | agacccgtgt | ctttccatgc | ccgagggcca | cgacgtcact  | atgcagggca  | 1680 |
| cagctgggctt | gggtttaa   | ggctcatctt | gatttatctt | agtaaatgtt  | ataaattatt  | 1740 |
| ttttagatct  | tgaattttat | ataaaaaata | ctttacctac | cctgatccac  | aaaaacctgat | 1800 |
| gttttaaatg  | tgcctttctt | tgaatttata | tgttttcaaa | taaaatctcc  | ctaaagcaat  | 1860 |
| attttaaaat  | nggtmaaaaa | aaaaaaaaaa | aaaaaaaaaa | ggcgcccgct  | ctagaggatc  | 1920 |
| caagcttacg  | tacgcgtgca | tacgacatca | tagnccgatc | cct         |             | 1963 |

&lt;210&gt; 34

&lt;211&gt; 1767

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 34

|             |             |            |            |             |             |      |
|-------------|-------------|------------|------------|-------------|-------------|------|
| cggcagcagc  | gcggctcgct  | cataggccga | acaaccaaac | agaaaagt    | aataaacagc  | 60   |
| ggacggaggg  | gcgcggcggt  | gcggagcgga | gcaagcagg  | gttcggcg    | attacctgta  | 120  |
| cccattcacc  | ggcggctacc  | ggcggcgccg | cgcagctgtc | aggcggagag  | accgcggcc   | 180  |
| aggaaatgaa  | ctgaagctgt  | ctgcagtaaa | acacagaagg | ctttaaatgt  | ttttcttgca  | 240  |
| taaaatttcaa | aagttttaag  | tagctgtctt | tgagaatagg | gaaggcagaa  | agctaatgtc  | 300  |
| tgctctcaaga | tacaggacag  | ctgtttgtct | atcaacctca | actgtgtgtg  | caactgagga  | 360  |
| acatgggtca  | agaaaactaa  | cacagccaag | tgccatgtct | ttgttccact  | ggctgtggat  | 420  |
| tttatggaaa  | ccctcgttaca | aatggcatgt | gttcagtatg | ctataaagaa  | catcttccaa  | 480  |
| gacagaatag  | tagtaattggt | agaataagcc | cacctgcaac | ctctgtcagt  | agtcgtctgt  | 540  |
| aatctttacc  | agtccaatgc  | acagatggca | gtgtgccaga | agcccagtc   | gcattagaact | 600  |
| ctacatcttc  | atctatgcag  | cccagccctg | tatcaaatca | gtcactttta  | tcagaatctg  | 660  |
| tagcatcttc  | tcaattggag  | agtagatctg | tggaacaagc | agtacctgaa  | acagaagatg  | 720  |
| tgcaggcttc  | agtatcacag  | acagcacagc | agccatctga | agagcaaaag  | aagtctctgt  | 780  |
| aaaaaccgaa  | acaaaaaaag  | aatcgctgtt | tcatgtgcag | gaagaaagt   | ggacttactg  | 840  |
| gggttggaat  | ccgggttgga  | aatgtttact | gtggtgtaca | ccgttactca  | gatgtcacca  | 900  |
| attgctcttca | agcttacaac  | gccgatgtct | ctgagaaaat | cagaaaagaa  | aatccagtag  | 960  |
| ttgttggtga  | aaagatccaa  | aagatttgaa | ctctctgtgg | aatacaaaa   | tcttgagcat  | 1020 |
| ctgcacaacta | aaaattgact  | tgaggttttt | tttttccatg | tcaattgggaa | tgtagagcag  | 1080 |
| tgtatcttct  | atgtcatcgg  | aagaatagat | ttttgtttgt | gtttgtttgt  | gaaaattgact | 1140 |
| ctgaacattt  | atttccattct | caatttctgt | ggctgaggag | acttaaaact  | tacaagtatt  | 1200 |
| atccttttaa  | gatcatttta  | atttttagtg | agtcgagagg | ctttttata   | caaacgtgca  | 1260 |
| gaaattttgc  | agggctgtga  | tttttccagt | attaaacatg | catgcattaa  | tcttgagatt  | 1320 |
| ttttcttca   | ttgtgtatgt  | atatatcgct | ttctcttgca | gcacgatttc  | ctttttgata  | 1380 |
| atgcccttta  | gggcacaaat  | agttatcagt | aactgaaatg | atcttaatac  | ttatggctgc  | 1440 |
| ttctgttttt  | tcattacaac  | aggttatcca | tatgttagca | tatagtttct  | ttgcaccacc  | 1500 |
| tatttatgtc  | tgaatcattt  | gtcacaaag  | agtggtgtgt | gatgagattg  | taagtttgtg  | 1560 |
| tgtttaaaact | tttttttgag  | cgagggaaga | aaaagctgta | tgcatttcat  | tgctgtctac  | 1620 |



|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| aggtttcttt | cagattatgt | tcatgggttt | gtgtgtat   | aatatgaaga | atgatctgaa | 1680 |
| gtaattgtgc | tgtatttatg | ttattacc   | agtctttgat | taaaaaaaa  | ggaaccacg  | 1740 |
| aaaaaaaaa  | aaaaaaaaa  | aaaaaaa    |            |            |            | 1767 |

<210> 35  
 <211> 1817  
 <212> DNA  
 <213> Homo sapiens  
 <220>  
 <221> SITE  
 <222> (1796)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (1797)  
 <223> n equals a,t,g, or c

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| <400> 35    |             |             |             |             |             |      |
| ggcagcaggg  | attattacat  | gccgataagg  | tatgaattgg  | agatggggatc | caaaattact  | 60   |
| gtacttagtg  | taatttttaa  | gctaattaca  | gggttggtgg  | tgaatttttt  | actttcgcaa  | 120  |
| aatgaaaca   | gcatttcac   | agaagttgtt  | ttatggcttt  | tcagacattt  | tattgtatat  | 180  |
| ctttggtgct  | ttttgcccat  | gaagtatctc  | ttttgtgtgt  | ttgtgtgtat  | gtgtattgat  | 240  |
| ctgctgataa  | tttcacagca  | ctcattgtca  | gggatcagca  | gaatcaggcc  | taggcagaga  | 300  |
| gctgacagct  | actctcagta  | cgtctctgtg  | tccttgttgc  | cacattatct  | ctgttgttta  | 360  |
| tttataagtt  | ggagaacttt  | ctgacctgta  | acaaagggtt  | gataggctag  | agacaattgg  | 420  |
| gagaggaatc  | ctctgctcta  | ttgtcacctg  | tgccctcact  | caaaatatgc  | tctgaaatca  | 480  |
| atatttaaat  | attaaatttt  | tatgggtgaat | gtatctcttt  | ttaaawgttaa | ttaaaagatt  | 540  |
| ggtcagccca  | cttaaatatt  | acaatgcagg  | acttactttg  | cgtattctct  | gtcttgtatt  | 600  |
| tttgcctttat | ttaagctagg  | ctagcctcca  | agctgggaagc | tgaattgmca  | gttgaaaaat  | 660  |
| aatgmcatgt  | atmcaaggta  | tgtttgaagg  | attgcagatg  | cagggggacc  | atatgctaaa  | 720  |
| ggagtgttgg  | aagctcactg  | cagaagatga  | caaaagcaga  | ctgatattga  | ttatttctgt  | 780  |
| aaatataaag  | tggaggcaca  | ggtgaagatt  | gccaaacctc  | atgaacagtt  | tggcacaata  | 840  |
| gacaggctgt  | caggccatgg  | cagttcamca  | gtgggcgtgc  | tgccctgtga  | ccaagtcatt  | 900  |
| tgttccagag  | gactacactt  | aaataccaca  | aaataaatct  | tccttgtcac  | tgatatcaca  | 960  |
| tggaatatga  | tggtgtcttt  | cagattttctg | gttgaattac  | cagccattaa  | catctggtga  | 1020 |
| tttgggttgt  | aaaattattt  | ttagttttgc  | ctgttcatat  | ttcatccaga  | aagcccaaac  | 1080 |
| aagatatatt  | ttccccacata | agaattgaag  | cagtaataatg | ccccgtccgg  | gagggaggcg  | 1140 |
| gggggcagcc  | cccccggcgc  | cagccggccc  | ktccgggagg  | tgagggggcg  | ctctgccygg  | 1200 |
| cygcacctac  | tggaagtgta  | ggagcccttc  | tgcccgccca  | ccaccgcgtc  | tggaagtggt  | 1260 |
| accacaacgc  | tcattgagaa  | cgggccatga  | tgacaatggc  | ggttttgtgg  | aatagaaaag  | 1320 |
| ggggaaaagt  | ggggaaaaga  | ttgagaaatc  | ggatgggtgc  | cggtgtctgt  | tagaaaagag  | 1380 |
| tagacatggg  | agacttttca  | ttttgtctgt  | tactaagaaa  | aattctctctg | ctctgggcatc | 1440 |
| ctgtgtatct  | gtgaccttac  | cccccaacct  | gtgctctctg  | aaacatgtgc  | tggttccact  | 1500 |
| cagggttaaa  | tggattaaag  | cggtgtcaag  | atgtgtcttt  | ttaaacagag  | gcttgaaggc  | 1560 |
| agcatgctcg  | ttaaagatga  | tcaccactcc  | ctaactctaa  | gtacccaggg  | acacaaaac   | 1620 |
| tcgggaaggc  | cgcaggggtc  | tctgctcagg  | aaaaccagag  | acctttgttc  | actgttttat  | 1680 |
| ctgctgacct  | tcctctcact  | attgtcctat  | gacctgccca  | aatccccctc  | tcgcagaaac  | 1740 |
| accacaagaat | gatcaataat  | aaaaawaaaa  | aaaaaaaaaa  | aaaaaaaaaa  | aatgttnaaa  | 1800 |
| aaaaaaaaaaa | aggggggg    |             |             |             |             | 1817 |

<210> 36  
 <211> 2488  
 <212> DNA  
 <213> Homo sapiens



&lt;222&gt; (27)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 37

```

ggtcgaccca cgcgtcgctt ttttntttt ttttttttt ttttttttt taagtctata 60
agcttttatg ataactttgt tttacagttc acaatgcatt ccacagattt agttcagtac 120
agcttaaaacc acaatggtaa aaattctcat ttgttaatta ataattcttt gcataacaat 180
gtttgataatt tgcaaaacaa caacattttt ggaagcatta gattcagtc c atagattctg 240
tgacaaaatt aactacagtc agtctgtgca atgaaattga tgttggagtt ctatgtgtgt 300
ggcatttcat gttgaaaaca gatggtagtg ctctagaaa tattttctct tctagcttat 360
gtgccttgga actacacatg tataaccaat gactgactct gaaatatcaa gcactgtggg 420
gtggctggaa ggtaaaggtc taagctttgt gaaacactat acatatataa tctatattta 480
ctatatattg caattaatat aacagtaaaa gtcaacaata acctagaaca taccagaaaa 540
gcaagctttg tcattctctg tttaccggta tgatctctgc taaacaaaaa tttcaattca 600
gaaaatctgc atcaatctac acggaccata cacagtgcac aaactgaaaa gggctttttt 660
tttttttttt ctagctccac catctctgca acttgccaag atgcggcaag actatctgca 720
acaaagtaaa atatacaggt ttttatctc accagtgcct cagataggaa aaagatatga 780
ttacggttta aatccatata tagcagctta caatacttaa gatgatgaac acatggcagt 840
caagacaggt aatttttctt cacaacagtg catggctaaa aataaagatc taacaacgat 900
ctgtgaaact gcaactgcaa gtcaagggtc gttctctcct gaccctcccc cgtataatca 960
aatgaataac ccctttaagg atgaactcct actaattatt ttgggcgttt tcatcagct 1020
ttgcgtctca atccagggat tttgtctgg attttagcca tagcatcttt aacattctta 1080
tttgcaagtc ctagataatg atctatctgt gcctgatgcc gttcataaat aacaggaaca 1140
ctgaagagtg aaatgagagc caaaatcagt agtgtcagac cattaaacaa ggcaccaaca 1200
taggtaaaata cccacatcaa cactgcaaac ttcaagaaat caactaaatc atcaactaag 1260
aagaggcgcc tgagttctct tatcgtcgag ttcaactgac caagagcaga attactgtac 1320
ttctgaacca actcctcga tatagcaact tcagattcca gatatgcct gactgggtgg 1380
ccttcatctg atttctgtga agcttgggac acacccttgt atatctaaa gctgatggtc 1440
acagagagca gggccaaggg aatgtaggct gttacgtcca caatgctgaa tactgtcaat 1500
gaaagcagca ggaataggct ggcaccaaac accactccag tcttcttaaa gctctccag 1560
tacaggaggt caacaactgc agaggagcgt atcacaggct cagatgcagc aggaagagca 1620
aaaagggtct catccaactg ccccgaggag cccctgcgct tgggcgcggc cggggtggag 1680
gggggcgcgg cgggagccgg ggctggcggg gtccacacgg gctctgcctg ggggctcacg 1740
ctggcgcggg ctctgtgcc

```

&lt;210&gt; 38

&lt;211&gt; 1158

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (826)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (842)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 38

```

caaggtcgcc ggtatcgata agctttgata tgaattcaa ccaatcactg ttatcagggc 60
agtcatccct ttgtccagcc atgcctcgtg atcagagaag cactgccatg acagatattg 120
ggcaaaactga tatatgccc ccaatactgg gtttgggtct cattctcgtt aatgcatgtg 180
ttaagggttt caltctaatc atctgcaaaa aagtgtattgt tgcctttaaa atgtgtacca 240
aactagctct ttacgaagaa tcttactaga ataaaatggc tcttgcacc tgatttttta 300
atcagcctat gtatatgtat cccattctgt atattatcac cttaatatat aacagcccca 360

```

|             |             |             |             |            |             |      |
|-------------|-------------|-------------|-------------|------------|-------------|------|
| attcctcttta | ttgagcattg  | gttgctaggg  | aaagcaaaaa  | gttaagagaa | aactactgag  | 420  |
| atcttctctc  | taattgtcct  | ctgtctgcct  | ctaactagct  | tagagatctt | ggacaagtcc  | 480  |
| tttaacagtt  | ctctgtcagc  | tgtctggact  | gtgaaataga  | gataaaagta | cctgtctawt  | 540  |
| tcaggccggg  | cacggtggtc  | cacgctctga  | atcccagcac  | tttgggaggc | ccaggagggtg | 600  |
| gggtcacgag  | gtcaggagat  | tgagaccatc  | ctggctaata  | cagtgaacc  | cggtttctac  | 660  |
| taaaaataca  | aaaaaattag  | cggggggttg  | tgggtgggcac | ctgtagtcct | agctacctgg  | 720  |
| gaggctgagg  | caggaggaatg | gcattgaaacc | cggaggcgga  | gcttgcatgt | agccgagatc  | 780  |
| acgcccagcg  | attgtgcacct | gagtgacaga  | gtgagacycc  | stctcnaaaa | aatwaaacct  | 840  |
| tnaaaaaaaa  | aaaaaaacaa  | ctgtctattt  | catagaggta  | ttgtgaggat | taaatgagat  | 900  |
| aattgttgat  | aagtaatttt  | gaaaagcata  | tcaatgtgca  | gctgtaaaat | tttattcata  | 960  |
| tttaccagcc  | aggcaagtag  | tattagctgt  | tataagtaaa  | ggtaaacatc | caagtgcata  | 1020 |
| tggtcagatg  | attattttta  | atatacattt  | tctcctgata  | tatatctctc | tagtttgaga  | 1080 |
| tctgatttgt  | tgaattcgat  | atcaagctta  | tcgataccgt  | cgacctcgag | ggggggcccc  | 1140 |
| gtaccacaatt | ggccctag    |             |             |            |             | 1158 |

<210> 39  
 <211> 2190  
 <212> DNA  
 <213> Homo sapiens  
 <220>  
 <221> SITE  
 <222> (1008)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (1026)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (1085)  
 <223> n equals a,t,g, or c

|             |             |
|-------------|-------------|
| <400> 39    |             |
| aggggccggg  | cggtgccggc  |
| caagccacaa  | aaagtacagg  |
| aacttgctcg  | actgagagac  |
| ttgaagaaca  | caaatagata  |
| aggagtgggt  | gctaagagag  |
| aagaggccgg  | taaaaaacgg  |
| aacaggcagag | gaagagagaga |
| agggtattcc  | tgctcatggga |
| ycagagtttg  | ggtttttttt  |
| tcattatttca | gtttagacat  |
| gcagtgccag  | attcctttaa  |
| attggcaaat  | gttgtaaaag  |
| gtggcaaaaca | tcacactctg  |
| aatgggtatg  | gctgtgttcc  |
| ttatagcaag  | ttatgtctct  |
| aatactttgt  | tttgktttcca |
| gcaagaattta | gaggattttaa |
| ttttgntatt  | tgattataaat |
| gctancacac  | agttgcttat  |
| tatcttctct  | atctcccaag  |
| tccttgacag  | tatatattatt |
| cgcccgagaa  | gatgacgttt  |
| acgaaggagaa | acgaagaata  |
| cacagaaggga | ggaaaggagg  |
| tattggaaaag | agagaggagaa |
| aagaagaatt  | cagaataaag  |
| aagagagaaa  | gttaaggaaa  |
| agcagaaaacg | acaggaggaag |
| gatgagtttg  | aagaataaatc |
| cttgctattt  | cattgtttgt  |
| ttccctctct  | atgcctctct  |
| gctttaccct  | atttaccctt  |
| aaatatatta  | ggctttatgg  |
| ccatcgataa  | tatgtagatg  |
| acaggcagct  | agcccttctg  |
| agtgtaata   | tttagagaat  |
| gaactgctta  | tgacatgtca  |
| tgaggancct  | aggaaccagt  |
| aggcctatgaa | gataattgta  |
| gtgaggcata  | tggtgtgatg  |
| ttggtatgtt  | ttgctgctat  |
| cctcccttga  | ctgatttcac  |

|             |             |            |             |             |             |      |
|-------------|-------------|------------|-------------|-------------|-------------|------|
| aggattttgtc | ttttttgtaa  | tacagctaaa | tttaaatagc  | ataaaattgg  | ataatcacct  | 1320 |
| tataaattggg | aaaaaatgcat | gtgtcttcat | tgtagtcaat  | atggggcgaga | agcagcctcc  | 1380 |
| acgatctttg  | ttttttgtat  | cttaggccat | cagtttaatt  | cagtgcagat  | tttaaacacc  | 1440 |
| ttttattttg  | cactctcttc  | cagatgaggc | aagttcatgc  | taggttttta  | ggggcgagaa  | 1500 |
| aaattgtttg  | gaactgttca  | ttttttata  | gagaggaatt  | aattccatt   | tttaacagtag | 1560 |
| agtgcttcctc | aactgaggtc  | tttcaggaa  | tcagttaacag | ctaaggcctc  | ttttgaatgc  | 1620 |
| ttttgtttca  | gaggaatct   | gtgagactwc | aaggaraaat  | gaggggaaatg | aaatagattc  | 1680 |
| taagctttata | arraaraawtc | rgcagtagt  | gttcctgctg  | tatgctagac  | taagtcgtag  | 1740 |
| cggaacagaga | cagagcagtg  | aaagacacag | tctttgtctc  | cattgagcta  | gttttttata  | 1800 |
| tgggagtgtg  | agatgataag  | caaaacagat | cattcaaaat  | gtgaycata   | taagtctcat  | 1860 |
| aaagaataaa  | agcagggccag | gcgtggtgct | cgccctgta   | atcccagcac  | tttggggaggc | 1920 |
| tgaaggcgaga | ggaatcacgag | gtcaggagtt | cggggcccgc  | ctggtcagca  | tggtggaacc  | 1980 |
| cgctctgtac  | taaaaatacag | aagggttgcc | cgggcggtgt  | gatggggcgc  | tgtggtcccg  | 2040 |
| gctgctcggg  | agtcgagggc  | aggggaatcg | cttgaaccgg  | ggggcgaggg  | tttcagttag  | 2100 |
| ccaaagcttga | gccactgcac  | tcacaactcg | gcaatagaac  | tagmttctgc  | tcaaaaaaaa  | 2160 |
| aaaaaaaaaa  | aaaaaaaaaa  | aaaaactcga |             |             |             | 2190 |

<210> 40  
 <211> 2576  
 <212> DNA  
 <213> Homo sapiens

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| <400> 40    |             |             |             |             |             |      |
| agctttctctg | taccttctct  | gcaggtagat  | gggacaaatg  | agtgctcgga  | tcagcgggag  | 60   |
| tgggaaattg  | aaatactaca  | aagatctgtt  | taatcctgat  | accaactaat  | ctccctttca  | 120  |
| agggaagctc  | tgggaagctg  | tacagctcat  | ttatttttaa  | actttttctg  | tttaacagaga | 180  |
| tcgtgtggta  | atctgaggat  | ttttattcta  | cgctgctctg  | acagatggaa  | aaactggaag  | 240  |
| aaactctgggc | taacctctgt  | tttttggaaa  | atttagtagac | ttgggtggtga | agaaactggg  | 300  |
| aggagtggaga | tattagctaa  | ctttgcatag  | ccacataatag | agcgtcgagc  | ctgcattcca  | 360  |
| ccaaagagga  | acccaaaggc  | ctgtggtggt  | cccagggtac  | atattcatgc  | cagaagtga   | 420  |
| gtgctctgggt | aattcgtttc  | ctgaaagtgt  | atcgcatact  | tgtactgggt  | taggtttttta | 480  |
| gaactctcagc | cataaaaaatg | ggcagaattt  | tccttgatca  | tatcgggtgt  | accgcctctg  | 540  |
| tttcttctg   | aaactgtgat  | acgatctctga | ccaaccgctc  | agaaactcat  | tccactcggt  | 600  |
| tcacaggcgcc | cactggcgaga | gcatttcttt  | ttacaagggt  | agtttaacctg | cagtagacag  | 660  |
| aaagtcaaga  | tcgggtcatg  | ctcactggcc  | gccacatggt  | tcgagatgtg  | agctgcacaaa | 720  |
| actgcaaatg  | caaaactggga | tggatctatg  | agtttgcacc  | tgaagacagc  | cagcgatata  | 780  |
| aggaaaggccg | cgtgatctctg | gaacgtgctc  | tagttcgaga  | gagtgagggc  | tttgaggagc  | 840  |
| atgtaccatc  | tgataaacctc | tgaagataca  | gagagaaatac | catcttttcc  | caggtctctc  | 900  |
| tcactgaaaa  | caaaaaatcta | cttacatata  | ctgtcacctt  | agcatcagag  | tcggattaat  | 960  |
| gaactcgagga | caaaagagctg | gtgagaattc  | aaagtggaa   | ctttctttct  | ttcttttttt  | 1020 |
| tcgttttaatt | tttgtatttt  | ccatccaaca  | cgagtggtga  | gagagaataat | tatgcagatg  | 1080 |
| tcgttaattt  | tttaccctat  | gtttacatct  | tgaggcgagca | gagtcgtctc  | cgacgtctgt  | 1140 |
| ggtagagctat | gtaaagaaaa  | aaatctgggc  | tgttagagtg  | aaaaagtggt  | ttttatgtca  | 1200 |
| attgtgaaaag | gaaaatgtta  | ggagtaggt   | ttttaaactt  | gggcttcatt  | tttaactttt  | 1260 |
| tttttttaaac | caagttattt  | cacttgattt  | gctagcttca  | gagaaagatg  | cagattctgt  | 1320 |
| gccagcgctc  | aaaggctcag  | tgttagcatg  | gcttgctgtg  | gcggtgtgct  | ccatattctg  | 1380 |
| ttggagatgc  | accgtagcac  | cagagcccat  | tcttctctgt  | cagtccttgc  | ccaaagatgt  | 1440 |
| caccattctct | agttatttgt  | caccacataa  | ttggtgttga  | ttgggaaactt | tttctgagat  | 1500 |
| gggacagaaac | tgttgggttg  | tcctttttcca | tgtaaactta  | gcatagtaat  | atataataag  | 1560 |
| taattagtgg  | atgctttttg  | tctgtgtgtg  | cttttataaa  | caccttataa  | aaagggagag  | 1620 |
| tatttgataa  | gcaatttttca | tagtagtaaa  | gttttttttc  | atctcttaaa  | ctaaatctgac | 1680 |
| catgcatata  | atattctttg  | tttaaatgaa  | agcatctgtg  | tgaaaaccgc  | agtggtgcat  | 1740 |
| ttgaaaaaca  | gttgaacaga  | atgtcaatgt  | gcattcatgc  | aaaaaaacat  | ttaactctgca | 1800 |
| tcgtgttttag | aaaaaggggga | aatgaagcaa  | cttgtctaaa  | aataactgctt | tcaaaagcat  | 1860 |
| ttagcccttt  | ccccctcagt  | tttgacttga  | ttttttgaga  | agtcgtgaga  | gcctaattagt | 1920 |
| ttccattcaaa | ggcctagatc  | tcttattttg  | catttttttc  | agctctctct  | tcagaagtct  | 1980 |
| agctgttgaa  | acgaaaactg  | tactttgtac  | cctcacatac  | aaagggatca  | aaattgacct  | 2040 |

|             |            |            |            |            |             |      |
|-------------|------------|------------|------------|------------|-------------|------|
| gggtgtatttt | tagccccc   | tttatgacat | tacacaatat | taaaatgtaa | atgtttcttt  | 2100 |
| accctaaacta | cttctagata | ttctagtatt | tgcttctggg | ggaattaaat | gacggtaaaa  | 2160 |
| ttggctaatt  | atttgaatga | atgaatggat | ggatgttttg | catgctcaat | ttctaggctcc | 2220 |
| tttgtctaga  | aaggaaat   | gcctcagttg | aattagttaa | atatctctgt | cggtgatatt  | 2280 |
| aaaagtga    | cttgagtaca | gttaagttcc | tcctatttgc | cactgggctg | ttgggtagaa  | 2340 |
| gcataaggtaa | ctgattaa   | aggatgata  | ctgcatttga | aataagtggg | cacaaactat  | 2400 |
| ccctttccca  | ccatggactc | aattcgagaa | caacagcatt | catttccatt | catttccata  | 2460 |
| ctggcttttg  | attatatgca | gattccatgt | agcatgcctt | acctacarca | ctatgtgcat  | 2520 |
| ttgtctgcac  | aataaagtat | attttgtctt | gcaaaaaaaa | aaaaaaaaaa | ctcgag      | 2576 |

<210> 41  
 <211> 1152  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (27)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (29)  
 <223> n equals a,t,g, or c

|             |             |             |            |             |             |      |
|-------------|-------------|-------------|------------|-------------|-------------|------|
| <400> 41    |             |             |            |             |             |      |
| ctcttcacatg | ctttaacctg  | tcttgngnc   | ccagcatctg | ccctacatga  | ggcactctct  | 60   |
| ttaggaggct  | ctgattctaa  | gagaacagaa  | tatttggaa  | gggcgtaagt  | agatattgag  | 120  |
| tgactttctc  | ctttctgcag  | gactggatgg  | tttccatgat | cgtaggacaga | gagtacatg   | 180  |
| tgccagtgga  | ggccgtcaga  | ttactgatac  | ttatccttaa | gtgagtccctg | ggaagagggg  | 240  |
| aggccagtgt  | ctcagacctt  | tgcccttcca  | gggtcatctc | ccctccacctg | tcacagctga  | 300  |
| ctcttccatc  | tgtgcaggtt  | gactgaggtc  | attcctgagt | tgcatgtatgt | tgagagggtt  | 360  |
| atatcttctg  | cttctctaac  | tcctccatact | ccctgtctct | ccactctcca  | yttaggagtt  | 420  |
| ttttgtgagt  | tatgtccttg  | ttgcttttgc  | ctctttttct | ttctagccctt | gattgtgcca  | 480  |
| gaagacaatg  | tcctctattca | cacaactctt  | ctgcttttct | gtgggcagga  | acatggaagg  | 540  |
| gggtgctgatg | gacgtggact  | gtgagagcgt  | ctaccccat  | gtgtaggcct  | ctaattgagg  | 600  |
| ccctggccct  | gctgtgggtg  | aattttctga  | ctggaaatga | gtggggctcc  | ttttacgttt  | 660  |
| ctttaaacac  | accctctcgg  | tttctctccc  | acatgtgctg | ctgccctact  | taaggctccc  | 720  |
| ccatctccgt  | ggaagtctct  | ctggtgtctc  | tttctctctc | tcagctctct  | ggggctttcc  | 780  |
| ttagctctgc  | acctctcttt  | ccctttatcat | ctccttgggc | catctctctcc | stctgtgggtc | 840  |
| atattacac   | ctcctgtttt  | ctcctgtgct  | tgagcccttt | ccctgtgtcc  | attccaccag  | 900  |
| actttctac   | ctgtagctgc  | agataagaac  | gatgggtgga | agagagcaac  | gccagagccc  | 960  |
| aggygccag   | aggactttct  | tccagcttct  | gctgtgctct | ctttgtggag  | agcaagcaaa  | 1020 |
| tcgagtcttt  | ctccaggtct  | acaaggcgaa  | gggcatggct | tcgaagtgca  | aaagatcat   | 1080 |
| catgaaacat  | catgaatcat  | gaatcattag  | gcgtcttccc | ttcgtctccc  | actttttttt  | 1140 |
| tttttttttt  | gg          |             |            |             |             | 1152 |

<210> 42  
 <211> 3853  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (210)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (3808)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (3819)  
 <223> n equals a,t,g, or c

<400> 42  
 gcgctcccaa cgtggtggag gatgagattg atcagtagcct cagcaaacag gacgsggaaga 60  
 tttaacagaag ccgagaccaca cagctatgcc gccacggcct ttgggggaaat cgcgtgcaactg 120  
 cgtccctcta gaggggggaag tttgttgccc tggagaacat cagctgcaag atlaagtcag 180  
 ggtgocgagg gcactcccggt ggccgaatgn actctgtact aagtgccagc cgagcgccat 240  
 cagcgtgaac agacagaagt cagggcatgt ggacaatata atgtttgaga atcacaccgt 300  
 cgcgtgaccgc ttctctgact tctggagaaa gacagggaaac cagcatatttg ggtacttata 360  
 cggacgggtac acggagcaca aagacattcc ccttgccatc agggctgaag tggctgcat 420  
 ttatagacca cctcagattg gtacacagaa cagcttgagg cttcttgagg atccaaaagc 480  
 tgaagtggct gaagaaatg ctgccaaact tggcctcgag aagggtggct ggatatttac 540  
 agacctcgct tcagaagata cccgaaaggg tacctgcgcg tacagtcgaa ataaggacac 600  
 ctatttccata agttcagaag agtgcatcac tgcaggagac ttccagaaca agcatcccaa 660  
 catgtgccgg ctctctccag acggacattt tggatccaag tttgttactg cagtggctac 720  
 aggtggtcct gacaaccaag tccactttga agggtaaccag gtgtccaatc agtgtattgce 780  
 actggtccgt gatgagtggt tgctgcccag caaggacgcc ccggagctgg ctacgccaa 840  
 gagtctagca gtgagcrgta cgtgccctgat gtgtttata aggaogtaga caagtttgge 900  
 aacagagatca cccagctggc cggcccccgt cctgtggagt atctcatcat agacaacaca 960  
 acaactttcc ccaaggaatc agtttacaact ttttctattt ccgcaaaatcc atttctatt 1020  
 gaaaaccggg atgtattggc tgagacacag gacttccata gcttggccac ctatttgtct 1080  
 cagaatayct catctgtgtt ctgtgatacc atctcagatt tccacctctt gctgttctgt 1140  
 gtccaccaat aagttatgcc tctgcaggac agcatcagct tgctgctgga ggccgtcgag 1200  
 accagaaatg aggagctcgc ccagacatgg aagaggtctg agcagtgagg caccatcgag 1260  
 cagctgtgca gcacagttgg cgggcagctc ccaggtctcc atgagtacgg agccgtcgag 1320  
 ggctccacac acacggccac tgcagccatg tgggcctgtc agcaetgcac gttcatgaac 1380  
 ggcacagca caggccactc cgagatgtgc agcctccca ggacctaggg cgcctgccct 1440  
 ctgctggcta ggaccgggcc cagcccagcc cttctgaag ccagaagcgt tgctgagtg 1500  
 gttccctgta actgcccact agtgggcagc cctggaggaa caaggggctg gctgtctcgg 1560  
 gctccctgac ccactgaagc ttctcagcac gtctctccct ggagagcggg cagcatggct 1620  
 ggtattctgc aggtcgaatg cagctctccag actggaaacg cagagcgctt cctcacagct 1680  
 aatctgttg acaagtcctc ccgcctgtgt gaaagacctc tgcctctcat gtggccactg 1740  
 gaattggggc gcacaggagg gggcsgatgc tgccaccacc cagcctctctc ttattttca 1800  
 gtgcttttgg tttggctctc ctgcccactc ctgggagact ctggtaacc 1860  
 cctgtctctg ctgcttggtg gcaggcacc tgccctgtgag ggccacttct cctctctagt 1920  
 agctgtgtgc tgggtggggc cagtggtgcc tggacacggc tccctctctg aggtctgag 1980  
 gtcggtttgc tgccctgccct cctctctcct cccgatgtcc aggtgggatt ttaagctgt 2040  
 ctatggttat aataacagat atcagtaatt cctgcccaga agacttttat ttatttttt 2100  
 ttaagataaa aactgcacaa aaggggagtg agagagacta gtttccatct cctccctcc 2160  
 ttttagtgag cccccagagt ttgtgtccag gtgatgagt tggagcgggg caccagtcag 2220  
 ttctcccttg aagtaaacct cagtgctgta gacttttcta ccaagccaca cagctgcag 2280  
 aactgcagat actgcccggg gagcaggagc agtgggtggc cctgccctga gcttgccct 2340  
 cgatggcctg ggtgggagct ctgggtcacc ggtgcagat ctctggcctg ccccaatcac 2400  
 ctgctctgct ccaactgacc gcttggtgta gggcgttagg ggctgtgct ctgtgaggg 2460  
 ccaatttggg cctccctggg gcactgcaca attgatagt taccaataga cggagactgg 2520  
 ggcactgaga acagacgctg gtgggttctc gcttgtgtgc tctgcgttcc tctgtggct 2580  
 ggccagggcg gggcctggct ctaaccggg agtggtgggg ctctcgatg cttcttggcc 2640  
 ttaatgatgg ccacatctgg gctgctctgc acccagggga gaggtggccc cagctgcaga 2700  
 ctgcttaggg acttctgtgt ccatctctgg gggtaagccc acgtgaccca cattcttggc 2760

|             |            |            |             |             |            |      |
|-------------|------------|------------|-------------|-------------|------------|------|
| actatgaaca  | gagaacattt | gcctgttgcc | ttctgaagt   | gtcagggcca  | tggctgacac | 2820 |
| ctccaggctc  | gcctggcatt | ggacaccaag | tggaggcccc  | aagcagctca  | tctgctcttg | 2880 |
| ggacaggggg  | ccagtgggtt | tgggtctggt | cacggcagag  | ctwtgttggg  | gggtcaggaa | 2940 |
| gggtggagag  | gagctggggt | gaagcggact | gctgaggatg  | caactccagc  | cttgcccaac | 3000 |
| gcgggctgtc  | tgctctccct | cctagcagct | gtcacactga  | agttttgtcc  | tctgctgtct | 3060 |
| cctctggctc  | tgagatgagc | tgtgagccca | ggtggcccaag | gcttctctga  | ttgcttccct | 3120 |
| gtgagctcaa  | ggccttcccc | caccactggg | cagaggctgg  | acagcacgga  | ctcttagaga | 3180 |
| gagccgcttt  | gccagcttct | ctcccaactg | ctcgctctta  | tcacacacgc  | tattatagtt | 3240 |
| tcctgtgtcc  | tcacaccaga | tttcccttac | tctgaagttc  | cggcattcac  | atcattcatg | 3300 |
| ttttcttttg  | tccttttagt | aaaggaaaag | cattggcgat  | ttgtctgatt  | ctggttttga | 3360 |
| gttactcttt  | gttcagtaat | gcactttatt | ttattgtcca  | aagagagtca  | gagctaaaga | 3420 |
| tacaggcttg  | gggggtgagc | ctgctgtgag | agttcaggcc  | ctgggaggct  | cagccacctc | 3480 |
| ctctgtgtgg  | aaggaggctc | cagcccccac | tcgcatcttc  | acctgccctt  | gggtgtgaca | 3540 |
| caccctctca  | tgctaccagc | accataatcc | agtgggggtg  | actgggtgca  | cacctgcccc | 3600 |
| gggtgaacaca | gcggctgcca | gtctctctgt | cccgagagga  | ggtgggctgt  | gccctggctc | 3660 |
| cctccaaaca  | gctgctcctg | ggacacaggt | gctcctgctt  | cggctctggt  | tcggctcaca | 3720 |
| gggtgtgcatc | actgggcttg | gatttgcatt | acattgaccc  | cagccctgca  | gtggaacctc | 3780 |
| ataaaagcgc  | ctgaagcaaa | aaaaaarnaa | aaaaactcng  | ggggggggccc | cgaaccaatt | 3840 |
| cgcccaaaag  | ggg        |            |             |             |            | 3853 |

&lt;210&gt; 43

&lt;211&gt; 556

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 43

|             |             |             |            |            |             |     |
|-------------|-------------|-------------|------------|------------|-------------|-----|
| cagccaccat  | gcctgcctga  | aacctttttt  | aggtaaaagt | gaattccatc | cttaaaagtt  | 60  |
| tctgttatat  | ccatttttagc | catttttctat | tgtctcccaa | agaattccaa | tcacaaaaac  | 120 |
| agctttgaac  | tcctcccttca | aaggaaacag  | tcgacttttc | taattagcat | ctaccattat  | 180 |
| ccccaaatct  | tatttttatc  | attgacttga  | aattttttcc | aattgtcttt | tttttttttt  | 240 |
| tttaaggctt  | agagcagagg  | tttactaggc  | caaagaaaag | gaatagctct | ctgttgacga  | 300 |
| gaggggtcct  | ggagaaatgg  | gttaccaccg  | ttgtcttatt | taaatgggtt | cccatcagat  | 360 |
| tttaattttt  | tcttctcttt  | gagagcttgg  | taataagaag | cacttaaatc | actccaaaga  | 420 |
| agacttttaa  | aaggggagcag | tgaaaaggtc  | ttataatatt | attgattgaa | ttagaagaata | 480 |
| ctagcttaatt | aagaatctga  | gtctaacaag  | cacagatttt | ttctttctgc | ttttaaatgt  | 540 |
| tggtttttaa  | aaaaag      |             |            |            |             | 556 |

&lt;210&gt; 44

&lt;211&gt; 2150

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 44

|            |            |            |            |             |            |     |
|------------|------------|------------|------------|-------------|------------|-----|
| gcggacgcgt | gggtgcgacc | acgcgtccgg | tttgaccgt  | cggtcgtgag  | tgagaggaag | 60  |
| gggaaggag  | aggtcccgaa | tagcggtcgc | cgaaatgttc | cggtgtgagc  | gcctggcgcc | 120 |
| gggtgctttg | aagcagaagc | tggtgccttc | ggtgcggacc | gtgtgcgtcc  | gaagcccgag | 180 |
| gcagaggaag | cggtccctga | gcaacttgtt | ccagcgatgg | catgttcttc  | tagaactcca | 240 |
| gatgacaaga | caaatggcga | gctctggtgc | atcagggggc | aaaactcgata | attctgtggt | 300 |
| agtccttatt | gtggggtctt | caacagttag | agctgggtgc | tatgctctca  | agactatgaa | 360 |
| agagagatga | aaaagatata | atgaaagaat | ttcagggtta | gggctgacac  | cagaaacaga | 420 |
| acagaaaaag | gcgcgcttat | ctgcttcaga | aggagaggaa | gttctccaag  | acaaggcgcc | 480 |
| aagtcatggt | cctttctctg | taattgggtg | aggcacagct | gcttttgcgt  | cagccagctc | 540 |
| catccgggct | cggtatctct | ggggccaggt | actgattgta | tctgaagatc  | ctgagctgcc | 600 |
| gtacatcgca | ctctctcttt | caaaagaact | gtgggtttca | gatgacccaa  | atgtcacaaa | 660 |
| gacactgcga | ttcaaacagt | ggaatggaaa | agagagaagc | atatatttcc  | agccaccttc | 720 |
| tttctatgtc | tctgctcagg | acctgctcca | tattgagaat | gggtggtgtg  | ctgtcctcac | 780 |



|             |            |             |             |            |             |      |
|-------------|------------|-------------|-------------|------------|-------------|------|
| tgggaagaag  | gtagtacagc | tggatgtgag  | agacaacatg  | gtgaaactta | atgatggctc  | 840  |
| tcaaaataacc | tatgaaaagt | gcttgattgc  | aacaggagggt | actccaagaa | gtctgtctgc  | 900  |
| catttgatagg | gctggagcag | aggTgaagag  | tagaacaacg  | cttttcagaa | agatgggaga  | 960  |
| ctttgaagc   | tgggagaaga | tttcacggga  | agtcaaatca  | attacgatta | tcggTggggg  | 1020 |
| cttccctggg  | agcgaactgg | ccTgtgctct  | tggcagaaag  | gctcgagcct | tgggcacaga  | 1080 |
| agtgattcaca | ctcttcccgc | agaaaaggaaa | tatgggaaag  | atctctcccc | aatcctctag  | 1140 |
| caactggacc  | tggaaaaaag | tcagacgaga  | gggggttaag  | gtgatgccca | gtgatattgt  | 1200 |
| gcaatccgtt  | aggatcagca | gtggcaagtt  | acttatcaag  | ctgaagacgc | tgagggaagt  | 1260 |
| agaaactgac  | cacatagtag | cagctgtggg  | cctggagccc  | aatgttgagt | tggccaagac  | 1320 |
| tggTggcctg  | gaaatagact | cagattttgg  | tggcttcogg  | gtaaatgcag | agctacacag  | 1380 |
| acgcctcaac  | atctgggtgg | caggagatgc  | tgcattgctc  | tacgatataa | agTtgggaag  | 1440 |
| gagcggggta  | gagcaccatg | atcacgctgt  | tgtgagtggg  | agattggctg | gagaaaaatat | 1500 |
| gactggagct  | gctaagcogt | actggcatca  | gtcaatgttc  | tggagtgaat | tgggccccga  | 1560 |
| tgTtggctat  | gaagctattg | gtcttTtgga  | cagtagtttg  | cccacagttg | gtgtttttgc  | 1620 |
| aaaaggcaat  | gcacaagaca | acccccaaatc | tgcacagag   | cagtcaggaa | ctggtatccg  | 1680 |
| atcacagagt  | gagacagagt | ccgaggccctc | agaaattact  | attcctccca | gcaccccggc  | 1740 |
| agttccacag  | gctcccgtcc | agggggaggga | ctacggcaaaa | gggtgtcatc | tctacctcag  | 1800 |
| ggacaagaag  | gtcgtggggg | ttgtgctatg  | gaacatcttt  | aaccgaatgc | caatagcaag  | 1860 |
| gaagatccatt | aaggacgggt | agcagcatga  | agatctcaat  | gaagtgcaca | aaatattcaa  | 1920 |
| catttcatgaa | gactgaagcc | ccacagtgga  | attggcaaac  | ccactgcagc | ccctgagagg  | 1980 |
| aggtTgaatg  | ggtaaaaggc | cattttttta  | ttagcagcag  | ttctctctgt | tatgagtTgt  | 2040 |
| aatgatcaag  | tcctttgtga | atattttcaa  | ctatgtaggt  | aaattcttaa | tgttcacata  | 2100 |
| gtgaataaaa  | tctgtattct | tctaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa |             | 2150 |

&lt;210&gt; 45

&lt;211&gt; 2008

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 45

|             |             |             |            |             |            |      |
|-------------|-------------|-------------|------------|-------------|------------|------|
| gccacgcgct  | ccgcccacgc  | gtccgcccac  | gcgtccggcg | gccgtggagt  | ttgtgacata | 60   |
| cgaggtgaca  | cccctcgagt  | cacttccctt  | caactccagc | tggagcgctc  | gcttggcttt | 120  |
| gggttcgttc  | tgcagccttc  | gcccgcctcc  | tagcctcagg | gccggactcc  | agccgacagc | 180  |
| ccagcccagc  | gcagctgcc   | gcagcccacc  | agccgcccag | ccgcccagcc  | ccgcacgaaa | 240  |
| cccgcccaga  | gcttctcagg  | agcccagacc  | atgaacaccg | aaatgtatca  | gaccccctag | 300  |
| gaggtggcgg  | tctacacagt  | gcacaatttc  | tccattctct | tcttctcttc  | tctgcttgga | 360  |
| ggggatgtgg  | tttccgttat  | gctggacaac  | agtgccctcc | gagccagcgt  | ggtggccata | 420  |
| gacaaacaaga | tcgaacaggc  | catggatctg  | gtgaagaatc | atctgagtga  | tggatggaga | 480  |
| gagggagtg   | agatcctgaa  | ggagcagatc  | cgagagctgg | tggagaagaa  | ctcccagcta | 540  |
| gagcgtgaga  | acacccgttt  | gaagacccctg | gcaagcccag | agcagcttga  | gaagtccagc | 600  |
| tctctgtctga | gccttgaaga  | gccagctccc  | gaatccccac | aagtgcgccg  | ggccccctgt | 660  |
| gggttctcgg  | tgttaagtgc  | tctgtctctc  | gggtgggcag | agccactaaa  | cttgttttac | 720  |
| ctagtctctt  | ccagtttgtt  | tttgctctcc  | caagcatcat | ctcacgagga  | gaactttaca | 780  |
| cctagaccac  | ctgggtgccaa | gagatgtcct  | aaggacatgg | ccacctgggt  | ccactccagc | 840  |
| gacagacccc  | tgaacaagag  | aggtctctgg  | agggctgagt | gcattggggc  | tagtaaccac | 900  |
| aagccagtga  | gcctctaatg  | ctactgcgcc  | ctggggcgct | ccagggcctg  | ggcaactctg | 960  |
| ctgcaactgg  | caaaggagaa  | gggtagtTtg  | aggtgtgaca | ccagtttgct  | ccagaaagtt | 1020 |
| ttaagggtct  | gtttctctatc | tccatggaca  | tcttccaacg | cttccactga  | caacgactgt | 1080 |
| tcttatgaag  | aagccacttc  | tgTtttaagc  | agaggcaacc | tctctctctc  | ctctctgttt | 1140 |
| tgtgaaggcag | gggacacaga  | tgggagagat  | tgagccaagt | cagcctctctg | tTggttaata | 1200 |
| Tggtataatg  | catgtggagt  | tgcacagccc  | agTgtgggat | tacagctttg  | ggatgacccg | 1260 |
| ttacaaagtt  | ctgttttggtt | agtatttgga  | tagtttttct | atatagccat  | aaatcgctat | 1320 |
| ataataccat  | agggctagat  | ctgtatctta  | gtgtatcgat | gtatatccat  | acacatccac | 1380 |
| ctacatgttg  | agggcctcaa  | ccagccttgg  | gagTattgac | tggtccctta  | ctctctatgg | 1440 |
| ctaggctttt  | gactgtgttc  | atttaccag   | ttgaccagct | ttgtctttta  | ggtaagtaa  | 1500 |
| gactcgagag  | taaaggcag   | gaggggggcc  | agcctctgaa | tgcggccacg  | gatgccttgc | 1560 |
| tgctgaaccc  | ctttcccacg  | ctgtccactg  | aaacgtgaag | tctctgtttg  | aatgccaaac | 1620 |

|            |             |            |            |            |            |      |
|------------|-------------|------------|------------|------------|------------|------|
| ccaccattca | ctgggtgcta  | ctacatagaa | tggggttgag | agaagatcag | tttgggcttc | 1680 |
| acagtgtcat | ttgaaaaacgt | tttttgtttt | gtttgtgaat | tattgtggaa | aactttcaag | 1740 |
| tgaacagaag | gatgggtgcc  | tactgtggat | gagggatgaa | caaggggatg | gctttgatcc | 1800 |
| aatggagcct | gggaggtgtg  | cccagaaagc | ttgtctgtag | cggtttttgt | gagagtgaa  | 1860 |
| actttccact | ttttgacacc  | ttatcctgat | gtatgggtcc | aggatttggg | ttttgatttt | 1920 |
| ccaaatgtag | cttgaatttt  | caataaactt | tgctctgttt | ttctaaaaat | aaaaaaaaaa | 1980 |
| aaaaaaaaag | tttgcctcat  | aggtcgac   |            |            |            | 2008 |

&lt;210&gt; 46

&lt;211&gt; 1474

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1452)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1453)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1460)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1463)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 46

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| gggttcgacc  | cacgcgtccg  | gggttcctaaa | ttatgtttac  | tttgatttga  | ttatatgttg  | 60   |
| gtatctccca  | aatataggtt  | aacttagcta  | tttaaatggt  | atctttttgac | atttaaaaag  | 120  |
| aataaagtac  | ctgtcaaatg  | twgcattgag  | gttgcagttg  | aatagaataa  | aagcttagga  | 180  |
| tgtcaaaaaa  | taatatagag  | aaatattata  | agatttttatg | attatctctg  | amgtttttga  | 240  |
| tgcataaagg  | aaatatgctg  | aatagttctt  | ccaaaaataa  | ttatttccct  | caatatttta  | 300  |
| ttttagagcca | tgtaatttta  | agagaacaga  | aaataactgc  | aatcaaaaag  | atggtttta   | 360  |
| atcaatcaaa  | gtggcacaac  | agaattgata  | agatctttat  | aacaatcaat  | tggttgatat  | 420  |
| taaaatattg  | attttaattg  | atcttttcaa  | ttaaaaatctt | tagggcctgt  | aactcataaa  | 480  |
| atcagcatcc  | accacaatat  | atggtcatta  | ttggtttgta  | agcatagatc  | accattgact  | 540  |
| cctacctgga  | gagacatgct  | tatttctaaa  | aatccagtag  | tttctttgca  | ttctcagtag  | 600  |
| tacacgttgt  | atataatat   | atgtaacaaa  | tttggtagtt  | ttcagtagtg  | gtgatgtcct  | 660  |
| ttgggggtta  | tttatcttgc  | tggtccatag  | gaggggtaca  | ctaccccaag  | aatcaagaca  | 720  |
| ttctgagtct  | agttctagtt  | ctagctctgc  | cactgaagag  | ccaccttacc  | tggggcaagt  | 780  |
| tagccattgt  | ctcccgatca  | tgtttaccac  | ccatgaaagg  | actcgtcggg  | ttgatgtttc  | 840  |
| catataagct  | aatgagtta   | tttaaatagt  | actcttgta   | ctggattgaa  | aaacaccatg  | 900  |
| catctgtatg  | gataattcat  | aaatgttgc   | ctctttttta  | atgatacaac  | cctaaaagtg  | 960  |
| actgaattgc  | ccaagtgcct  | gaacatggca  | gaggtagtta  | ctcyattttt  | gcagttttgtg | 1020 |
| cacttaaaaa  | ttctcactag  | gattgttact  | ttactgggga  | aaaaagatga  | gggtaaaact  | 1080 |
| cctcccaagg  | aattaaaaata | ctctgtagaag | ccatggcttg  | cttttataat  | gtggaaatca  | 1140 |
| tttgattttgc | tgtaatctac  | gcagatccct  | ccttttttga  | gggggaaatg  | atttgcatca  | 1200 |
| tggttttttt  | ttcataatgct | tttacttctc  | gtttggatca  | gttgatgata  | aatgtacatt  | 1260 |
| ttgtgtactt  | tgctgtgcc   | gttagaattt  | atcttccata  | aagtatttct  | ccatttgagt  | 1320 |
| ctaattgatg  | atactttgcc  | taggtctttc  | caaaaattaa  | tttatgtaaa  | tgtctatttt  | 1380 |

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| atataaaata | tgattaaaaa | aaaaaaaaaa | aaaaaaaaaa | aaactcgagg | gggggcccgg | 1440 |
| taccaaatc  | gnnctatagn | canacggggg | taca       |            |            | 1474 |

<210> 47  
 <211> 1893  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (4)  
 <223> n equals a,t,g, or c

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| <400> 47    |             |             |             |             |             |      |
| tcctacttc   | gtccctgaca  | cccaggcctt  | ctgcccaccat | ctccccctgtc | atccgccaaac | 60   |
| tgcccaccag  | tgcccgtctc  | attgtcatca  | tcccaggac   | agtgatcgat  | ggccttgatt  | 120  |
| tgtctgaagaa | ggaacaccca  | ggggcccggg  | atgggattcg  | gtacctggag  | gcagagctta  | 180  |
| aaaaaggaaaa | caggtaacatt | cgctgccaga  | aagaggtggg  | aaagagcttt  | gagcggcata  | 240  |
| agctgaagag  | gcaggatgca  | gatgcctgga  | ctctctataa  | gatcctagac  | agctgcaaac  | 300  |
| agctgactct  | ggccccgggg  | gcagggtgag  | aggatccgag  | tggcatggtg  | accatcatca  | 360  |
| caggcctctcc | actggacaac  | cccagctgct  | tttcaggccc  | catgcaggca  | ggcctgcagg  | 420  |
| ccgtgcccca  | cgccagtggt  | gacatcaaga  | atgttctgga  | cttctacaag  | cagtggaagg  | 480  |
| aaattggttg  | atactgaccc  | ccaggccctg  | cagtggggct  | gactccagat  | ctctctctgc  | 540  |
| ctccctggca  | gccaggacca  | gcacctgtag  | tcaccccacc  | acacgcagac  | tcatgcacgc  | 600  |
| acacaggagg  | gaggcctagc  | tgctcagagg  | ctgcaggagg  | ggcccaggag  | cgggctggga  | 660  |
| gggtgggggt  | cccttgttgc  | caagacgtta  | ggaaagcgag  | gaaagtgctt  | ggattaggag  | 720  |
| agctctgtgg  | ggccctggcc  | agccttctgt  | cctcagctcc  | cctgctgtct  | ccaggggacg  | 780  |
| gtggtaggca  | tggttacctg  | catttccactg | gaatgggttc  | ttggatctct  | gaggggaagg  | 840  |
| aacagcaaaa  | gaggcccttc  | ttctctaccc  | aagatgcagg  | gtgggtgggg  | ccaggagttt  | 900  |
| ggacctctca  | ggctctgggg  | gaagagctgg  | gtaatacctg  | gtgtctgagt  | gattctctgc  | 960  |
| agacccttcc  | cctctccaag  | gatcacccat  | cctcctttca  | gcccccttta  | tggggaccag  | 1020 |
| gcagctctgg  | agccagccac  | aggggctgtt  | agagaagcaa  | ggcctggagt  | ggcctgcacc  | 1080 |
| gagtagcagg  | gtcagggttc  | gtgtgctcct  | cctcctgctg  | caggggctgc  | acatccatt   | 1140 |
| gccccacttc  | tgctttgtgt  | ctccctctgt  | ctagcttcca  | gggcaggagg  | caggccccac  | 1200 |
| ctagggtctg  | agggcagctg  | gcctgtgccca | gcacgggtctc | ctgtgcccac  | cagcccacac  | 1260 |
| gggtgctgtc  | tttgtgtctt  | tggtgctgtt  | gctgggacag  | aatgggatgc  | caggaagaga  | 1320 |
| agaaaagggg  | tgacgtctga  | ggccaccacc  | ccccttctca  | tctaaggagg  | ggctgaagac  | 1380 |
| aaagggccgg  | cattcagtgg  | gcagcagaaa  | ggagaggctc  | cttgaagctg  | ctcagtcaga  | 1440 |
| ggccccctgc  | cctccttttg  | ccttcccgag  | gactgaagac  | ctgaaggggc  | tggtctttgg  | 1500 |
| agtgtggaag  | tgaatatctg  | ggagcagaga  | tcattgaatg  | ctcaggggcg  | tgaatggcgc  | 1560 |
| accaagagca  | gggtgtgtgt  | tgaggaggctg | cagccaggat  | tgctctcagct | cctccccctc  | 1620 |
| aggctgggag  | gatagcacag  | gctaggggct  | cggggtggag  | ggctctcagct | ctgctgcccc  | 1680 |
| caccocagta  | ctagcctagc  | ttcccaggct  | gtggcttaga  | ggatagttgg  | cttctctgct  | 1740 |
| ctctctctca  | aaatagcaag  | ctctgggaaat | ctgggggtga  | gtggagtcac  | ccccctccca  | 1800 |
| gttgctggca  | gagactgaga  | ctaaagcatc  | acttaataaa  | ccccccaagc  | ccaaaaaaaa  | 1860 |
| aaaaaaaaaa  | aaaaaacctt  | gggggggggg  | ccc         |             |             | 1893 |

<210> 48  
 <211> 2418  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (138)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (171)  
 <223> n equals a,t,g, or c

<400> 48  
 gctcgtgccg ctgttgaccg cactgagcag tgctaagtgt tggtttagtg gatgttcgtg 60  
 gaattctgtga cccatccaag ggcgtccttt ggagccagtg gagcctgcg ggcgcattetra 120  
 gggggcagaat gctgctanca ctggaactcg ggaatctcgcc ttattctcaa ntagecaaggc 180  
 atctcgacaa gcatgggtcta rgtctgggtgg ccagcttgcc artacctgag ccggtcgggtg 240  
 catctgcctc tgaggggaccg tectcaccga gctcctgcat cccctgagtg ttgatcagga 300  
 ggcgtccaca gcaattgttct cgctctgaa tgatgcttct ttctgtgttg gagcctggcg 360  
 aagtgtgttt ttcaagccct ttactttttt ttccaagtg gggtaggagc ttttggcagt 420  
 gtttacttta cctagatggc ttatataatc cagtaagaga tgcacaagata aaattgtctg 480  
 ggttggtaca gaagcatggc ggccctccaga ctgaccattt ggttgccctt tagattttgt 540  
 aaggatgcgg tgctggggag gtgggtgcttc cctacccctt agaaatgctg ccttccaaact 600  
 accactctcc cagatgtgac ccttgogatt atttccctcg aggtttgagg atgaagataa 660  
 gtggaggga aagagagtaa ctaatagggg atgaaatata gcagaagcta gaagaagcg 720  
 gtgaggtgag agagatgcac ctgcacgttt tcttcaacag caccagggtga ttcagcata 780  
 tctcaattac ctttccactat tctgttatat aagatcgttt acttgcataa tatatcatca 840  
 atttgacata ttcttaaaac tagagggtgt gagaagcaca gcaataggaa gtctctccac 900  
 aaactagggg aacacaaatg ggggtcattca cgtgcctgga ctgtcactat gtggctgtca 960  
 cgtgaagtgc tgggtttgat ttccatttca gccagtggtt agctgataag ccagtgccag 1020  
 catccagcat gagcagatct cggggagact ggggaagtct cagcgtttact ccttcccttc 1080  
 ccttcagtat aagccagctgc cagcatccag cgtgascaga cgtcggggag actgggaagt 1140  
 ctccgatgtt actgcctgct ttcccttctg gtgaggggct tctcttgctt tctcttgctt 1200  
 ctgttagtgg acgaggtctt ccaaggaagt gctttgcaca ctttctttgc tctcttttac 1260  
 agtctttgtc tttgcagcaa gcaaatgaaa ttaagccact ttgggataat gaacattcag 1320  
 tataattctca ctttgtctca ttttggatct cactgttgtc ttataaaaaa tggcacattt 1380  
 tacaaagttag ttatttctta ttatacttct tgctggagag tgccttgaaa taaatgtga 1440  
 gagtattctg gtactctgtg ttccagatgc atgaaattgg gtgaggaata accctagtct 1500  
 tggaaatctt gtgaagcata ggggtattgc aaggcaaatg ggaactaaca catcttgcca 1560  
 tttgaatcag ggtctccagt ttctagaaaa ggcagacact ggttgggacc aaagtctcca 1620  
 tggcacatga ctgaagactg tgggtctgtg gtgtgcggag tccacrgaag cctcggggag 1680  
 gtggagctgc tctctccatt cgtgcaggac gtgatctgaa aacatgtaga gaagatgagt 1740  
 tgaggacagc ttctctaagg caatgtgatg tctttgcttt cttattcttc tttctcggg 1800  
 ttgttagttt tgaagagtgg aggagctagg ggtccagaa agaattctac acatgtgttg 1860  
 aagacattga tgtcatagg agcggggagc tgcattccct tctgggctgt tctgtcctaa 1920  
 tctcagtatg aacagaccag gcggaaaagt tgggtggcaa gcagctctgtg tgcttccccg 1980  
 ctgatggaga acgttgcgtt gttcaacata gggcctcatg ggtgtagccg catggcagac 2040  
 ccatggctgg ccagctgcgc tgttgccgtc tgtcttcagt aactgctgct cgtttaactg 2100  
 tctattcttg atactacggg tgtgtttttt tacaaacaggt atgttttttg tccagaataa 2160  
 tgtattgctt tctcatatt ttttgcataa tgtattgtca acatgggtca tttaaagtcc 2220  
 tttatgaacc ataacctgct gtggtagcct tgtacatgtg tgattctgtg tctttattc 2280  
 cagtggtgca tatgtgcccc tctgtattct ttgagaagtg cggaaatagg tgcctctacc 2340  
 acctgtctct aatgtaacag taaaagtttt cacttttttc tcagaaaaaa aaaaaaaaaa 2400  
 aaaaaaaaaa aactcgag 2418

<210> 49  
 <211> 1159  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (584)

<223> n equals a,t,g, or c

<400> 49

|             |             |            |             |             |             |      |
|-------------|-------------|------------|-------------|-------------|-------------|------|
| ggtgcgtggcc | gctctagaac  | tagtggatcc | cccgggctgc  | aggaattcgg  | cacgagctga  | 60   |
| agccgggggac | gcgggggggct | gaggaatgct | gaggccaaagt | ctgagcgctg  | gcccattgcc  | 120  |
| tgccctcccg  | tcctgctctg  | tgccccatg  | tcagtgccct  | cctgggttag  | gagacccgga  | 180  |
| ccggagacaa  | ccgagcgccct | gcagacccca | gagcgggctgg | gaacagtgtc  | tggaatgggg  | 240  |
| ccttttgca   | tcgcccggcc  | aagcctctcc | aaaatggatt  | ccactgggtg  | gggtgaggac  | 300  |
| ctggagcaag  | gtgcgcggcg  | acgccccac  | aggggtccca  | tggtttcaggc | tcctgcccgtg | 360  |
| gcctttcttt  | ctggactgca  | gctcagcttt | gttgctgtgt  | caatctactg  | attgtttctt  | 420  |
| ctggaatttg  | gggtccaagg  | ccctgactat | gaccaggatg  | ccccagtcct  | ttgctctaac  | 480  |
| ccggctacag  | aagtgacaac  | caggscacac | agaaccacag  | acaggggaccc | ttcamcccca  | 540  |
| cggttagccc  | aggggctcta  | gagctcacta | gcccataaga  | gggnctagac  | aagattcttt  | 600  |
| tcacaaatc   | aaaactcctg  | gcagttcctc | agggacaggc  | cttgagcagc  | agtgaatcag  | 660  |
| atgggggaat  | ttggggggct  | cacaaaggga | caggtacaaa  | caattcgctc  | gggggtcaga  | 720  |
| ggaggcaatt  | ctcagctgag  | cccaggcctg | agacccttgg  | tgctttaact  | ccaccactca  | 780  |
| agggcttttcg | tgatctgact  | ttctctctcc | ttccactctc  | tcactgtctc  | ctgaacaage  | 840  |
| aaaaacacatt | ctcgtctcca  | agctttgcc  | tggtttctcc  | agcacctctc  | ctgcttttgt  | 900  |
| ctgcctatca  | aacaccccac  | ttttaaagtc | cccgctcaaa  | aaatctccta  | ccccgtgggt  | 960  |
| ttctctatct  | ttctccacca  | acctctgtct | tgccactctc  | agggcagggtg | gaagctcttc  | 1020 |
| ttttcacaaa  | acaattata   | aacctcttta | agttctactg  | agttgtcccc  | tggtctatgg  | 1080 |
| ttctgaccca  | gaccagtagg  | tgcttaataa | atgttaatgg  | attcaattta  | aaaaaaaaaa  | 1140 |
| aaaaaaactc  | gtaggggggg  |            |             |             |             | 1159 |

<210> 50

<211> 2792

<212> DNA

<213> Homo sapiens

<400> 50

|             |             |             |             |              |              |      |
|-------------|-------------|-------------|-------------|--------------|--------------|------|
| gcaggaaagt  | gcactctatca | taagtgtgca  | aattgatgaa  | ttctaaaaac   | ttttattgtac  | 60   |
| ctgttttagca | cmtagattga  | cactgaacat  | aactaacaac  | cagaaatctc   | cgtgtactccc  | 120  |
| cttccgtgtaa | ctaccccctgc | gcccgaccaa  | atcactctct  | tctaacagca   | gtccattttgtg | 180  |
| tgactagcttt | ttttaattgta | aaagaatgaa  | atctacagca  | tgattattcatt | tgcatctggc   | 240  |
| ttctgcccac  | caacattata  | tttgtgggat  | tcrtttgtac  | agttgcataat  | tagtttgccag  | 300  |
| atccctcact  | ctcatttctta | tatgggtatta | tattgcataa  | acgtaccaca   | ctttatccaa   | 360  |
| ctactgttaa  | atattttgtc  | attttctact  | tggggggtgat | ttcaaatagt   | gctgctatga   | 420  |
| acattcttgt  | aaatgtcttc  | tggtgaacat  | atgcaacaca  | tatatgtgggt  | gtgtgtgggt   | 480  |
| cccgaggagg  | gcatttccctg | gtcataaaca  | atgcgtgtgt  | tcagggtttag  | tacagataaa   | 540  |
| tgccaaacag  | gtttccaaag  | gttttgtgcc  | actttacata  | cctgccattta  | ttgaaaagga   | 600  |
| gtctctgttg  | ctccacattg  | tcaccaatac  | ttgatatttt  | ctgctttttt   | ttctctttaa   | 660  |
| acgctactag  | tggtgtgtgca | gtgatattgc  | aatgtggttt  | taatttgcat   | cttccctgtg   | 720  |
| acaacctgta  | ttactgttaag | ccacttgtaa  | atgtgatatta | aattcatata   | aagatatagt   | 780  |
| agcaaaacgc  | atactaggtt  | actttcgtat  | ccagaaagt   | tagatagaat   | gatttctatg   | 840  |
| taagctttta  | ctgtgtagtc  | tgagtcgatg  | aatattgatt  | acaaaacaaa   | cactgtgtagg  | 900  |
| tgagttacaa  | tacctcaatt  | ataattomaa  | attcatgttg  | gtttagctca   | atatttttcca  | 960  |
| aatatttttt  | gcattgcaatt | ttcactctct  | ttctgagtag  | tttcaggtag   | tttgtattgtg  | 1020 |
| ttccagcagtc | agtttaggttg | ccattgtttg  | gaagcacaca  | ttccagtagc   | tgccacatga   | 1080 |
| tgatatgaca  | cccacatacc  | ccccatttca  | cattttgtca  | gaagtgcatc   | gtttacacta   | 1140 |
| actttgcccag | tagaaattgta | ctcccaattt  | cccacggact  | tattcttgat   | aatctctcca   | 1200 |
| ctgaagcatca | acaggttttg  | aatctctgtta | gaattagtgt  | ttttactact   | tttttaatttt  | 1260 |
| acataaaatt  | caagatttag  | taatactttt  | atttataaaa  | tgaacacaa    | cttttctctc   | 1320 |
| ctcttaccaca | catgttagtc  | cagcagaggg  | ggaaagcatt  | ggmcccgccc   | caaaatcata   | 1380 |
| aacgctttcca | attaaactat  | aatatttgct  | ggcatgttgc  | catataaatt   | tttgtctcca   | 1440 |
| ttatctctgg  | ttgcttttat  | aaacccatag  | gtcactgaag  | cccacttttt   | agacaaagac   | 1500 |
| tattttctccc | ccaaaagtca  | agggaaatat  | aaaaaatgaa  | attagtgtat   | aagaaatgaa   | 1560 |
| gtcaattaat  | acawtcattt  | tgtcttaatt  | atttaaagtc  | cagttttttc   | cctccagcaa   | 1620 |

|            |             |             |             |            |             |      |
|------------|-------------|-------------|-------------|------------|-------------|------|
| acctgaaaat | acactatcct  | ccagctatca  | gaattatatt  | gagatctact | cacatttatg  | 1680 |
| atgatgttca | gagatttctca | ttgggaagga  | aaaggcacac  | gctgcggcgg | tcttgcatga  | 1740 |
| ctctgtttgt | ttggaaattc  | aatttgttca  | ttgtgttttg  | ggctctctgg | tttgctcagg  | 1800 |
| ctgggctctg | ggctctctgg  | aattctctcag | gttcccagca  | ctccaaagcc | aagctcacct  | 1860 |
| ctctatcaca | caccctacag  | gagaagcatt  | aggggtccg   | actacgtggg | tttcatagct  | 1920 |
| gtggaaaagc | caaaagggag  | actcctgaag  | aaaggcggtg  | aagactgtga | agagcgggtc  | 1980 |
| aggaagtatg | gcacagcact  | gctactcctg  | tgggcacagg  | gacagcatgt | ctccagcgac  | 2040 |
| ygccaccttg | tttaatacat  | gggaactcac  | tgaaattcat  | tctgtatttt | gcccgcgaaag | 2100 |
| ttttaaagmt | ttcatccaca  | gtcaggaatt  | aaactatata  | caatgagagc | ctcacacatt  | 2160 |
| caaggatgta | ctaagcacta  | caggcctcac  | agaaacagag  | atcccatctt | ggagttttca  | 2220 |
| gtaccacatg | ggagataaag  | gggtttgaac  | atgaaatgac  | aaaaacaaca | gcaagaagaa  | 2280 |
| aattcttgtc | ctttttcatt  | actatcagac  | tcaataataat | gtcttggtct | ttacattaca  | 2340 |
| ttcattcttc | aaccatttgt  | gtctggcttc  | cacttctctc  | acttcacca  | catggctctg  | 2400 |
| ccaaaggaag | ccogtgatct  | ctaggccatc  | actttaattg  | atctytctac | aacattttac  | 2460 |
| ctggttgtta | agccctcctt  | acaacattct  | tctctctttg  | tttttatagc | tccatctctc  | 2520 |
| ctgcttcttt | aaacttgataa | tgcatacttg  | atttttctat  | ttgtattttc | ataaaccaat  | 2580 |
| taatacacag | ataaaatgac  | tgtatatcaa  | accatgtttg  | tatagaaaaa | atggattttg  | 2640 |
| gatgcctctc | atatgttaatt | agttctatta  | aacatattaa  | ttgtatttgt | taatttgtca  | 2700 |
| gggttttgac | agaattttgt  | ttacaagtaa  | taaaaaattt  | atctccaatt | ttcaaaaaaa  | 2760 |
| aaaaaaaag  | tcgrrggg    | gcgaatttat  | ta          |            |             | 2792 |

<210> 51

<211> 1921

<212> DNA

<213> Homo sapiens

<400> 51

|             |             |             |            |             |             |      |
|-------------|-------------|-------------|------------|-------------|-------------|------|
| gcagacccca  | aagcgctgt   | gggtgctgtg  | agtgagccgg | cctgtgccca  | tgccctggag  | 60   |
| gctttgggta  | ctctcctgcy  | cccacggta   | ctggacctgc | tagtctccag  | cccagctttt  | 120  |
| ccccaacag   | tgggttccct  | gagtcacag   | cctatgcccc | tagagcgttt  | tgggcgcgcg  | 180  |
| ttccccctt   | ccccagggag  | cgctctagaa  | gagtatgggt | ccttctatgt  | agggggctct  | 240  |
| aaggccagcc  | ctgacccaga  | ccttgaccca  | gacctgagtc | ggctgtctct  | ggggtgggga  | 300  |
| ccaggtcaac  | ccttctcctc  | ctgctgtcca  | gatacaggga | agactcagga  | tgaggggtgc  | 360  |
| cggtctggac  | ggctaaaggg  | acgaagacga  | tatctggtag | agagggccag  | agatggccgc  | 420  |
| gtggtagggc  | tgctggcagg  | cacactgggt  | gtagcccaac | accgtgaggg  | actggccca   | 480  |
| ttgcggaaac  | tgactcaggg  | tgtctggcaa  | cgtaactatg | tgttggccct  | ggggcgccgc  | 540  |
| acccttgcga  | agcttgccca  | cttccctgag  | gtggatgtct | ttgtgtctatt | agcctgtcct  | 600  |
| ctgggtgtct  | tagcccccac  | agcttctggt  | agcttcttcc | gctctatagt  | gcacacatgt  | 660  |
| gagctggaa   | ctgctgtcaa  | ccctgctgtg  | ccactccag  | gcctggctcc  | ccactcaca   | 720  |
| catattggcg  | acttattgtc  | tggctctccc  | ttccactggg | ctctcccaac  | acctgaggtca | 780  |
| gagctgtggg  | aaacccagca  | cgtgtcactc  | attactggag | atctccgacc  | cccactgtgc  | 840  |
| tggaaagtcat | caaatgatca  | tggaaagctt  | gctctgaccc | cacggccaaa  | gctggagctc  | 900  |
| gctgagagca  | gtcctgcagc  | ctcattcctt  | agttcccggg | gctggcaagg  | gtcgagccgc  | 960  |
| cgctcggtgc  | agacagatc   | gacagaagct  | gtgagtgaa  | gacgagggaa  | gctcatcgcc  | 1020 |
| tatgagagtg  | agggaaagcg  | ctgatacatt  | gtggggctgt | agacatagat  | ggaacttatga | 1080 |
| atggctgtcta | ggactctttag | tgtctcctgc  | accaaactcc | ctatccccctg | ccaagatctc  | 1140 |
| tgaaggagcc  | tggaaaggag  | gagagcaggg  | agcccttcc  | aggataggat  | ccgtctctgt  | 1200 |
| cctgtctctg  | cactgggaca  | agctcagcac  | atgcccagta | atgcctgtgt  | tttggctgtat | 1260 |
| ggaaataaag  | gcttagggag  | ttccctgagg  | cctctggagc | catctgtctt  | cttagaggga  | 1320 |
| gcccaggagc  | tttggccaat  | cccagttccc  | aggtctcagt | tgagggctgt  | tcttctgtcaa | 1380 |
| aaggcaggtg  | ctagacagtc  | tagaacaggg  | tttctcaaac | ctgtactctg  | catttggggc  | 1440 |
| cagataattc  | tttgtttgtg  | ggctgtctgg  | tgtatggtag | gggtgtcagc  | agcatctcct  | 1500 |
| gcctctgccc  | actagacatc  | agaaagcactc | ccccagttgt | gacaaacaaa  | aatattctcca | 1560 |
| gaccttgcca  | aatgttatct  | gtgggggaaa  | attgccctca | attgagaacc  | actgggtctag | 1620 |
| ctagaccctg  | actgtccagt  | acagtagacca | ctaaatacat | gtggcttaaac | ttaaatttaa  | 1680 |
| gttaattaa   | attaaaagct  | cagtttctca  | gtcacattag | tcattcaagt  | gttcagacag  | 1740 |
| ccacatgagg  | ggacagtgc   | gctacaggat  | atgccatcat | ggcagaaagt  | tctrttgggt  | 1800 |

|             |            |            |            |            |            |      |
|-------------|------------|------------|------------|------------|------------|------|
| ggacagyggt  | ggtctatact | gactcttatt | tctcagggag | atcacagcaa | cctaataaaa | 1860 |
| ccagataacct | tttcaaaaaa | aaaaaaaaac | aaaaaaaaaa | aaaaaaaaaa | aaaaactcga | 1920 |
| g           |            |            |            |            |            | 1921 |

<210> 52  
 <211> 1494  
 <212> DNA  
 <213> Homo sapiens

|             |            |             |             |             |             |      |
|-------------|------------|-------------|-------------|-------------|-------------|------|
| <400> 52    |            |             |             |             |             |      |
| ccaaggagca  | gcctcaagcc | cagagaagac  | tgaggctacg  | gggaacttcc  | cttgctgcag  | 60   |
| gctgtgtgtaa | cactttaag  | gccccaggct  | tccactgcga  | agcaggactt  | ggcacatgag  | 120  |
| caccacaccc  | acagcaccat | caggcagcac  | ccatgggctc  | caccagccca  | gctctgtccc  | 180  |
| tcccctagg   | aaagatcaca | ctgaagtctt  | caagtcccca  | gcaaccaagt  | tgggggtggt  | 240  |
| ttccttctct  | ttcaggcagc | caggcccatg  | gctggaaggc  | cgggctggag  | gcccagggaa  | 300  |
| tcggttactg  | atgtggccac | cctattccca  | catggggagc  | accaggagcc  | ctggagcccc  | 360  |
| ttccacccaa  | ttccatctcg | caggagagca  | gggtctgggc  | tcctgctcca  | ccgctgggtc  | 420  |
| agcagacccc  | tcctccgcgg | gtccacacct  | gccttttgaa  | gaagagcccg  | cataatgagt  | 480  |
| ggagcggcaga | cagctatatt | tagtggtgct  | tcgacactca  | cgaaccgcga  | gcgtgggcgc  | 540  |
| tggatcttgc  | ccagctgcga | gctcccccca  | ccaggactgt  | ggttctctcag | ttctctctgc  | 600  |
| cagccccggc  | tcactctcag | gcaaaagctat | agacatggta  | gatctcatcg  | gggagggtct  | 660  |
| cctgctctct  | ctcggccagg | aggctgaggc  | ctgcactgca  | gatgatcctg  | cggaccacgt  | 720  |
| caaggtcccg  | gcacacgcgt | ctgtccacgt  | cgctccagaat | caagccctcc  | tgggccatgt  | 780  |
| tgtctttgat  | gacgatgatg | ccgttggggc  | ggaggctgcc  | cttgacgcgc  | cgcaggaaat  | 840  |
| cggccaggtg  | ctgatccggt | agggtggcctg | gggagggcag  | aggcaaggtt  | accagggcac  | 900  |
| catgcgaagc  | gatgtcggtc | ctggatggga  | tgtacttctc  | agccccgctg  | agccctcat   | 960  |
| ttgtgccag   | catcttctgc | gcacacatgg  | cctcattggc  | tctcagctcc  | gagagattta  | 1020 |
| ctaaacccca  | attcatagaa | taggaaactg  | tggctcagag  | gtcatctacc  | taaaagacaca | 1080 |
| ttaagccaga  | atttgacttg | acgggatggc  | ttacaagtcc  | tgctctgata  | ctacacacca  | 1140 |
| tgatgccccca | ccctgcggag | gcttggatcg  | gaggagtga   | cctggggcca  | gagtttgsga  | 1200 |
| ttgcaggagc  | tcccagagag | actgccttcc  | ctacactaga  | agtccccaca  | aaggctctctg | 1260 |
| agaccacaaa  | atacaaggcc | agacctagag  | tgtccacagc  | aggcctgtgc  | tgcccgcctc  | 1320 |
| aggaggtggc  | cgactgtcgc | tagcggcctg  | ccgccacgct  | agcagcccg   | ggcacagcgc  | 1380 |
| tcgaggtgtg  | ctgttaggct | aagagggtgc  | agggctagac  | acgaagctta  | aactattcat  | 1440 |
| cttattaaaa  | ataaaacctt | tataaaactc  | caaaaaaaa   | aaaaaaaaac  | tcga        | 1494 |

<210> 53  
 <211> 1700  
 <212> DNA  
 <213> Homo sapiens

|            |            |            |             |             |             |     |
|------------|------------|------------|-------------|-------------|-------------|-----|
| <400> 53   |            |            |             |             |             |     |
| gaattcggca | cgagatgctc | cagaacttag | ggcctagaga  | tagcaaaccc  | gaattccaaa  | 60  |
| atgtctctgc | tacttatcaa | ccgtatgtgg | ccttgggttaa | gtactgaacc  | tcttttggct  | 120 |
| tcagcttcat | catttatgtg | ggaattcagc | tcacataga   | ttctygtatc  | tctcgcgctc  | 180 |
| tttcagcaat | aaaagtggag | cacattaatt | tgatgtgaat  | gtccccatgg  | ccgtatccac  | 240 |
| actgaatctg | gactttttaa | agtgctctgt | atttgtttac  | atgttttagag | gatttttgatg | 300 |
| actggctcag | tgccaatgac | tcagaggctt | aggcccatct  | gtgggtttgg  | tttacgcaaa  | 360 |
| ttgttatctt | ttgtgggatt | cgctcagctc | tgaattgttt  | gcaagacaga  | caaaagaagc  | 420 |
| ccacataacc | acccttatcc | ttctctgtca | cagaaaagaa  | accattttcc  | attctacaag  | 480 |
| cataaacctc | tcaagtgggt | aaatataga  | aaagcagtg   | tcataataac  | atcgttaagt  | 540 |
| tttttacacc | agcaacatcg | agcttggctc | agatctgcca  | tggagcagc   | ctagcttaag  | 600 |
| agctagaggc | ctgtgtgctt | caaaagactt | catgtgagat  | ttttgtgtac  | tttactcagg  | 660 |
| aaagtgtgaa | tctctaaaaa | ataataataa | taagcacatg  | ttttcatgat  | tcattaatct  | 720 |
| tcctttttgt | tcctataaac | atttcttgg  | tgtgctccca  | agctttctcg  | gagctctcac  | 780 |
| aaaggagagt | agccaggagt | aatgagagga | gggtcagggt  | ggcacttcca  | aaagctggag  | 840 |

|             |             |            |             |            |            |      |
|-------------|-------------|------------|-------------|------------|------------|------|
| agacccttct  | gaggaccctc  | caccatcagc | ggggcctcag  | gggacccctg | cctaacactg | 900  |
| gtgtggctgc  | tgggcccagc  | gctcacctgc | acagaactcc  | tccctgctcc | cactctgaag | 960  |
| ggcgtttcag  | aattgactca  | tttccactgc | agtcgtccag  | aaccctgtta | ataaggagct | 1020 |
| gtgtttatatt | acatctgggg  | ctgtgtgcat | ctttacaaat  | ggagacacac | tattcaaagg | 1080 |
| gaagattctct | aaagtaaggga | gcgtggtact | tattctttgt  | ttgaacatt  | ccgatttgtt | 1140 |
| gtcaggcctt  | atttgttttt  | tgtaatgcat | tgtatcggag  | gcttaagtgt | tgtggaatca | 1200 |
| gtccccacgt  | gttgaagac   | aagtacctct | tttctgacac  | ttcttactac | ttttacaggt | 1260 |
| agtaaacagct | acaaatactc  | tcccagaagt | ggaggagacac | catttgtggg | tggggagaaa | 1320 |
| gaggggagagg | ttctatgtcc  | acttgcctca | ttaagaatgt  | taagggttaa | gaaagtgtat | 1380 |
| tctgtcttga  | gaatttctca  | agctttacat | ttttttttca  | ctgaagaaaa | taaacaaact | 1440 |
| ctaaagggtg  | tgcataatata | acatttcttt | agatctttac  | ctaaawggaa | tttttaata  | 1500 |
| ttattgtgtg  | atgtaaaatt  | tgtttctgtg | gagttttgtt  | tgttttgcct | tttctagact | 1560 |
| gccatgtgca  | agtaaaattc  | ctgcctctgc | catgttaaaa  | tttttaata  | tttttaatat | 1620 |
| taataaacag  | agaaaaacaa  | atttaaaaaa | aaaaaaaact  | cgtagggggg | gtcccgtac  | 1680 |
| ccaatcgctc  | ctsatgagtg  |            |             |            |            | 1700 |

&lt;210&gt; 54

&lt;211&gt; 1177

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1071)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1164)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 54

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| gaattcggga  | cgaagattcgg | cgaagatagg  | gaataaggaa  | gcacaggagt  | aggggagaag  | 60   |
| gaagcacagg  | agtaggggag  | atatacacgc  | gtcaggataa  | gggggaaaag  | gcggtggttg  | 120  |
| cgcaaggagt  | gaaacaagat  | gtgagagaca  | aggggtaggg  | aagaaatggg  | gcagcggtta  | 180  |
| gggttcagaag | cgcatagacc  | gtggcggacg  | ggcaatgcga  | ggggcacaga  | aaggaactga  | 240  |
| gggggtgggct | atttaaggag  | atggctctca  | gccctctctt  | ttctgcgtag  | gtctctctct  | 300  |
| ccaggcccgcg | cgcgggatgt  | tgcctccggaa | accagcccag  | tctaggctgg  | atgatgacc   | 360  |
| acctctctct  | acgctgtctc  | aagaactacca | gaatgtccct  | ggaattgaga  | aggttgatga  | 420  |
| tgtctgtgaaa | agactctttg  | ctttggaaat  | ggccaacaag  | aaggagatgc  | taaaaactaa  | 480  |
| cgaaagaacag | tttatgaaag  | agatgtgttc  | aaacccagag  | gacacccagat | ccctggaggc  | 540  |
| tgaattattt  | gccttctctg  | tcaagatccg  | cagtttatgaa | gaacacttgg  | agaaaacatcg | 600  |
| aaaggacaaa  | gccacacaac  | gctatctgct  | aatgagcatt  | gaccagagga  | aaaagatgct  | 660  |
| caaaaacctc  | cgtaacacca  | actatgatgt  | ctttgagaag  | atatgctggg  | ggctgggaat  | 720  |
| tgagtacacg  | ttccccctct  | tgtattacgc  | aagagccac   | cgccgattcg  | tgaaccaaga  | 780  |
| ggctctgtgc  | attcgggttt  | tccaggagac  | tcaaaaagct  | aagaagcgaa  | gaaagcctt   | 840  |
| aaaggctgca  | gcagcagccc  | aaaaacaagc  | aaagcggagg  | aaccacagca  | gccctgccaa  | 900  |
| atgcatacca  | aagacactca  | aagacagcca  | ataaattctg  | ttcaatcatt  | tctttctgct  | 960  |
| ttagaagaatg | ataggagaga  | tgtatgggct  | ctttttggcc  | tgaagaggag  | aggaatttta  | 1020 |
| ttctttcatt  | cagctytag   | atcccagagk  | tttctgagc   | aragtcctcg  | nclyaccac   | 1080 |
| cagaggatcac | acacttatga  | catgcctccc  | acacattcat  | acaaacaagt  | acctgttcat  | 1140 |
| ccagggtggtg | tgtgttctagt | ggngggcaag  | acagagc     |             |             | 1177 |

&lt;210&gt; 55

&lt;211&gt; 1574

&lt;212&gt; DNA



&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (457)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 55

|             |            |             |            |             |             |      |
|-------------|------------|-------------|------------|-------------|-------------|------|
| gtacggattc  | cggggtcgac | ccacgcgtcc  | ggcggcgccg | acggcgacat  | ggagagcggg  | 60   |
| gcctacggcg  | cggccaaggc | ggcgcgctcc  | ttcgacctgc | ggcgcttcc   | gacgcgacgc  | 120  |
| cagggtggtg  | cgcgcgcgt  | gtgcttggct  | ttcgcttga  | tcgtgtttctc | ctgcattcat  | 180  |
| ggcgagggtc  | acagcaatgc | ccacgagctc  | aagcagatgt | actgcgtgtt  | caaccgcaac  | 240  |
| gaggatgcct  | gccgctatgg | cagtgcctac  | ggggtgctgg | ccttctctggc | ctcggccttc  | 300  |
| ttcttggtgg  | tcgacgcgta | tttccccag   | atcagcaacg | ccactgacgc  | caagtacctc  | 360  |
| gtcattggtg  | acctgctctt | ctcagctctc  | tggaccttcc | tgtgtgttgt  | tggtttctgc  | 420  |
| ttctccacca  | accagtgggc | agtcaccaac  | cgaagnacg  | tgtgtgtggg  | ggcgcactct  | 480  |
| gtgaggggcag | ccateactct | cagcttcttt  | tcattcttct | cctgggggtg  | gctggcctcc  | 540  |
| ctggcctacc  | agcgctacaa | ggctggcggtg | gacgacttca | tccagaatta  | cgttgacccc  | 600  |
| actcoggacc  | ccaacactgc | ctacgcctcc  | taccaggtg  | catctgtgga  | caactaccaa  | 660  |
| cagccacctc  | tcaccacaga | cgcggagacc  | accgagggtc | accagccgcc  | ccctgtgtac  | 720  |
| tgagcggcgg  | ttagcgtggg | aagggggaca  | gagagggccc | tcccctctgc  | ctctggacttt | 780  |
| cccatgagcc  | tcctggaact | gccagcccc   | ctctttcacc | tgttccatcc  | tgtgcagctg  | 840  |
| acacacagct  | aaggagctcc | atagcctggc  | gggggctggc | agagccacac  | cccaagtggc  | 900  |
| tgtgccccaga | gggcttcagt | cagcygtcca  | ctcctccagg | gcacttttag  | gaaagggttt  | 960  |
| ttagctagtgt | tttttctctg | cttttaatga  | cctcagcccc | gcctgcagtg  | cttagaagcc  | 1020 |
| agcaggtgtc  | catgtgtctc | tgacaaagtc  | ctcagcttcc | ccccggcccc  | gggtcagcgc  | 1080 |
| tgggagccgc  | tattatctgc | gttctctgcc  | aaagactcgt | ggggggccac  | acaacctgcc  | 1140 |
| tgtgcagcgg  | agccggacca | ggctcttctg  | tcctcactca | ggttttgctc  | ccctgtgccc  | 1200 |
| actgtctgat  | gatctggggg | ccaccaacct  | gtgcgcgtgg | ccctctgggt  | gcctcccggt  | 1260 |
| gtgtgagggc  | ggggctgggt | ctcatggcac  | ttcctctgtg | ctcccaacct  | tgggcagcgg  | 1320 |
| gaagggtctt  | gcctgacaac | accagctttt  | atgtaaatat | tctgcagttg  | ttacttagga  | 1380 |
| agcctggggg  | gggcaggggt | gccccatggc  | tcaccaagct | tgtctgtgcc  | gagtgattta  | 1440 |
| taaaatcgtg  | ggggagatgc | cgggcctggg  | atgctgtttg | gagacggaat  | aaatgttttc  | 1500 |
| tcattcaaaa  | aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | 1560 |
| aaaaaagggc  | ggcc       |             |            |             |             | 1574 |

&lt;210&gt; 56

&lt;211&gt; 2735

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (66)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 56

|            |             |            |             |            |             |     |
|------------|-------------|------------|-------------|------------|-------------|-----|
| gaattcggca | cgagtggagc  | tgtggtcaac | tcgagtgtgg  | cagaagcaca | cagcttgcca  | 60  |
| gcgcanaaa  | gcattgctgt  | gatggactgt | catcgactc   | acttgtcaga | agaggtaactg | 120 |
| gctatgctta | tgctccttag  | cactttgcct | gcagtgtgtc  | cagcaggctg | tactccaaa   | 180 |
| attcagccat | tagatgtatg  | catcaaaaga | actgtcaaga  | acttctctga | taaaaaatgg  | 240 |
| aagggaacag | ctcgggaaat  | ggcagatact | gcattgtgatt | ctgatgtcct | gcttcaagctg | 300 |
| gtgcctgtct | ggctgggtga  | agtgcctagg | gtcaattggg  | actgtccaga | gctagtccag  | 360 |
| cgctcctctc | tgggtggctag | tggttctgct | ggccccgatg  | gcaacattaa | ctcactaca   | 420 |
| agaaatgctg | acatgcagga  | ggagctaaat | gcctcctcag  | aggagcctag | gaagctgag   | 480 |
| ggggaacatt | ctgagctctc  | camtccamga | cccagatcat  | ctctgaaga  | gacaattgag  | 540 |
| cctgaaagtc | ttcaccagct  | ctttgaggg  | gaaagtgaga  | ccgagctttt | ctatggctttt | 600 |

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| gaagaagctg  | acctagatct  | gatggagatt  | tgagtgttgg  | ggatcatgagg | gggtgtggag  | 660  |
| tgggggtggg  | gaacaatctg  | agggagggtg  | aaggggctta  | gggaaaaggg  | ggcataccag  | 720  |
| gtgggttatt  | tggtttctat  | tttttaattt  | tataccacca  | ctccccctgt  | aagttgactt  | 780  |
| acactccctc  | gtggatttgt  | ggattaatta  | ggaaaaccaa  | tagtaatacac | gtctgagcca  | 840  |
| aggagctggc  | ccattggtca  | ttcactctcg  | ctaaaaacag  | gttttttgga  | cttttttttt  | 900  |
| ttttaaattt  | aaatcaactgt | gtttgtgtatt | tttctgacaa  | aattaaagaaa | agaaaaaaa   | 960  |
| attattttgtg | ggcaaatgtt  | aaattttttt  | gtttcccttt  | ttacctcaat  | gtatcatag   | 1020 |
| tactgggttt  | ttttgtttgt  | tttattgtgt  | ggccaatgtc  | tttgggcatg  | atgctatcta  | 1080 |
| atcattgtta  | atgtgagaac  | atttctgaag  | atggggaaga  | caaatattgt  | agctcaca    | 1140 |
| ctgggtttatt | atatatatgg  | ataaaaaact  | tttttcattg  | tggtcttaac  | actttttat   | 1200 |
| aaaaatgaaa  | atggaaaaaa  | agtcccactg  | aactctctct  | tccttctcct  | ttcttttct   | 1260 |
| tcctctccca  | gagatgttgg  | tttctacagc  | aaccttagat  | ataaaattgt  | ggctttaaaa  | 1320 |
| atgcatgaaa  | ccacttttaa  | ttatccagaa  | tgaatagatt  | tgctttttcc  | tcaccacctt  | 1380 |
| ccctccaaaa  | catgacataa  | acaatatttt  | ttgcaactgt  | gatccttggc  | ccctttcccc  | 1440 |
| attctcaaca  | ccatccatcc  | ctctggacaa  | aggatcatat  | agggtgtatt  | agcaagcaag  | 1500 |
| agatactgaa  | gcgatcaaac  | agtttttaggg | tggaaagccat | tcccagtttg  | agtcttcac   | 1560 |
| ctgtaagccc  | ccaggggcag  | tcctgtcttt  | actgaacttc  | atcctgttag  | atggagagca  | 1620 |
| tgctgtttaa  | agggattact  | ggctctacag  | ccaggagcta  | attgttcaag  | aagttgtgaa  | 1680 |
| ctttaaaaaa  | acaagaccac  | ttgttgaaat  | ccagcgtgct  | ctgtggcttt  | ccctatttt   | 1740 |
| tcttaatact  | tagggaagaa  | tctgacagga  | agaagcgcac  | aggggtgtgc  | acaaagaaaa  | 1800 |
| tgacatgaat  | ctttattttt  | cactgccagc  | ttcaaggaaa  | gaaaattttt  | tctacaattt  | 1860 |
| gcattgagga  | tttttttaat  | tgtatgtact  | catggttgta  | aaacaaaacg  | ctactgtacc  | 1920 |
| tacagagaaa  | aggagcaaaa  | aaccaagtct  | tctgtttatc  | ctgaggcttt  | ccacaattgt  | 1980 |
| ccctctctgt  | gagccaagga  | ggcaaatgac  | acaagcttgt  | aaatggttcg  | tctttaaaaa  | 2040 |
| gtacataagt  | ggaacattta  | ataaaatgag  | gggaaatgga  | tttataaaat  | tggttttttt  | 2100 |
| ctaggtgacc  | ctgtttaata  | ggcttttcac  | gactggggaa  | tgctcaagat  | gtgaggggac  | 2160 |
| tggtggtaca  | gggtgtgacat | ttgtttaccac | ccattttctcc | caccccaccc  | tgcttttttg  | 2220 |
| tttgtttgtt  | ttttcatccc  | ccagcacact  | ataatatagt  | gaactggaaa  | agtcctctcc  | 2280 |
| agaaaacagct | tgggccagctt | tgtgaacctt  | tgacatctga  | aaacaaccaa  | ggatccatct  | 2340 |
| gggctctctc  | tcgccagctt  | tttgctgtat  | gccattttat  | tgacagacaa  | tggaacttga  | 2400 |
| agtcagcctt  | tgccctttgag | aaagtccaag  | aactatgggt  | ggtcacgtct  | atctacaacc  | 2460 |
| taactctatt  | cttttgtagt  | ctctgcagca  | gccacagcct  | tagcagagct  | gggtttctct  | 2520 |
| tcttctgcac  | acgattgact  | ttcttgatgg  | gtaatttttt  | ttgaagattat | accaacagtg  | 2580 |
| gatcagctgg  | gttttggcca  | ggaaagtgtc  | tttgtggact  | ctgcctgact  | ggcttttagt  | 2640 |
| tagaaggaaa  | tttttttttg  | gttttgtttt  | ttataattca  | gtttaatcaa  | taaacaatgta | 2700 |
| tttattgact  | rttaaaaaaa  | aaaaaaaaaa  | aaagg       |             |             | 2735 |

<210> 57  
 <211> 2731  
 <212> DNA  
 <213> Homo sapiens  
  
 <220>  
 <221> SITE  
 <222> (579)  
 <223> n equals a,t,g, or c  
  
 <220>  
 <221> SITE  
 <222> (1532)  
 <223> n equals a,t,g, or c  
  
 <220>  
 <221> SITE  
 <222> (2362)  
 <223> n equals a,t,g, or c

```

<400> 57
gctggggaag gtgcaggggg cctgggagac tgcctctccg aagcgggtgaa accctcacc 60
ttccgtccct cccagccacc ctccctaaaa ctccccctga cagaggggtgg cagccccagg 120
ctctttgcat aatcctctgg cttcgtgtgc ttaccaccagc accagcggagc aggggaagggc 180
agagaaggcc cccatgtcga cactcctctc ccatccgcag cagccctctc cctctctgcg 240
ccaggccatc aagaataagg gccgcagagt cagagatcta caggatcccc cgcctccaaat 300
ggccccggag attcagcctc catcccaacca ctctcccccc gagcagcggg cctcgtctcta 360
cgaggacgca ctctacactg ttctgcaccg cctgggtcat cctgagccca accatgtgac 420
ggaggcctct gagctgtctg gatacctgca ggaggccttc cactgggagc ccgaggagca 480
ccagcagaca ctgcagcggg tcaggggagct tgagaagcca atatctttgtc tgaaggcaac 540
agtgaacacg gcccaaggga ttctgggcaa agatgtcant ggggttcagc acccctactg 600
cctgctgggc attgagcagg ggggtargtg gccagggggc agccccgggt ccggcgatcg 660
gcagaaggct gtgggtgagg acaccatccc cgaggaggag acccaccgca cgcaggtcat 720
caccagacca ctcaacccc tctgggacga gaccttcac ctggagtttg aggacatcac 780
caatgcgagc ttctcatctg acatgtggga cctggacact gtggagttctg tccgacagaa 840
gcttggggag ctccagcgtc tgcattgggt tcgcaggatc tttaagagg ccggaagga 900
caaggccak gacgaacttc tggggaacgt ggttctgagg ctgcagggtc tgaagctcac 960
gggtgggga tccccgcctg tcaggacctg cgctgccagc aggaccagtg gtacccccctg 1020
gaaccccgca ctgagacctc ccagaccga gccacgtgcc acctccagt ccaactctac 1080
cataagcga gacgcacttc ggccagccgc tcgcagccsa gctacaccgt gcaactccac 1140
ctcctgacg cactgtgttc ccacgaggtc acccagcaga aggggggaag cacctcctgg 1200
gacgggtcgc tgagtcccca ggctgccacc gtccctcttct tgcacgccac acagaaggac 1260
ctatccgact tccaccagt catggcgca ggtgtggcct acagccgctc ctaccagagc 1320
ctggagttcc ccagcagctc cctcctgca cccatcacc gaactcgagt ccagttgatc 1380
cagggtcgcc tcaaggcaga acagcaggag gagctggcgc cctcattcag cctcctgtga 1440
cctacggcct ctccctacc ggaggttccg ctctgtcttc cccctctctg tctcggactc 1500
cccagccgg gtgcagcttc ttctcagggt cntggtacag atgtgcaaga tgaagcctgt 1560
tggaagaactg tgcctcaaca ccgccccatt gccccagctg gtgactgagg cctctgcagc 1620
tgccaccact gaatgggttc acctgaagca gcagcaccat caacccatgg tgcagggcat 1680
cccgcagcca ggcaaggcct tgcctgggct ggtacaggat gtcattggcg acctgcacca 1740
gtgccagcgc acatgggaca agatcttcca caataccctc aagatccacc tcttctccat 1800
ggctttccgg gatgtgcagt ggctgggtgc caagcgggtg caggaccaca cgacggttgt 1860
gggtgatgta gtgtccccag agatgggcga gagtctgttc cagctctaca tcagcctcaa 1920
ggagctctgc cagctgcgca tgagctcctc agagagggat ggagtcctgg cctcggataa 1980
tttccaccgc ttgttccagc cggccatccc ctctcggtg cagaagacgt acaacgagge 2040
cctggcgcg gtgcagcgcg ctgtgcagat ggatgagctg gtgccctgg gtgaactgac 2100
caagcagcgc acatcagcgg tggatctatc cactctgctt gccagatcca gccacactgc 2160
ccggcagctg gactggccag acccagagga ggcttctatc attaccgtca agtttgtgga 2220
ggacacctgt cgcctggccc ttggtgtact gacctctata aagrmccagg ccccgagct 2280
ctcttcaggc cagaaggacc aaggccaggc agccaacatg ctgtgtgtgg ttgtgaatga 2340
catggagcag ctgcggctgc tngatcgcca agttgccgc ccaagctggca tgggaggccc 2400
tggaagcagc ggtagggggc gtgctggagc aggggcagct gcagaacacg ctgcatcccc 2460
agctgcagag cgcgtggccc gggctgggccc atgagatccc caetggcctg cgcacctggt 2520
ccagacagt ggaggtgggc atcgccaagc acatccagaa actggtgggc gtccaggagt 2580
ctgtcctgcc tgaggtgcc attctgcccc tgatgaagtt cctggaggtg gagctttgct 2640
acatgaacac caacttggtg caggagaact tcagcagcct cctgacctg ctctggaccc 2700
acacactcac agtgctggtg gaggcggccg c
2731

```

```

<210> 58
<211> 1468
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (561)
<223> n equals a,t,g, or c

```

<400> 58  
gcttccatat cagaagcctt gacttaggag tcagacctgg gttcaaaagg tagtttccatc 60  
atttcttagc tatgtgacta tgggggtgtt acttaacctc tctgagattt ctttcccttga 120  
tttataatgt ggcatagaaca atacctgcoct tgcagtgttg catgtggtga tgtgtcttgc 180  
ttggagcttc ctgtggaggtt tgcctcttca tgggttcaatt caotcctttt ttctattcat 240  
ttcttcatgt gctgtatcat glacctagga acatatagct tagtagctta gcagctttagc 300  
actaggcgtg gggcacaaaa ggggtggagag ggtgtgtgct tagccttttg wggctggtaa 360  
ctctgggccc agagttagg ggaatgattt gactggcgag gcaagactctg ctgtgtccca 420  
ttgtaaacca gtcatagaag agatgcctgt cctgggtagg ggcctctctt tccttccggt 480  
agtgtcttga gcatgacctg gatgacaggc ctattgagca gtgacctggg acctgggagg 540  
gaaactaggg ctggagagca nctctgggag gcatgggga gggcgtaggt gtagtagtca 600  
ccatctctcc tcaaggacgt cttgggcaag ttgtctggcc ccattagcca gcaaccaggg 660  
aaatgtagct gcaggaaaa cactctgttt cctcggtatg tttttcttta ggctggtttc 720  
ctttacaagg tgcaattatg ttccatccca cgcaattcag taagtggcac ttttcagaga 780  
aactgtcttg gtgatcattt gggctgctgt gggccaggga gttgaggaga gaaggagagt 840  
agagcttcta ctgagtttag ttggtttgt gttccatgagc catttacaaa ctttgcacct 900  
gattgggctc agttgcagtt tctgttattt cctaccagc caagctgttg aagctgttga 960  
gcccggaaat atgttatcac tgaggcagat gacaaacct ctagtgtcta gaaaccaa 1020  
tgctccccgc gctgggtttt ccgttttttg tactgactgc ttatttggggc ttgatata 1080  
atggtgaaaa caggaaactgt ttatttttag tgataagaaa ccaacattat gacaagaaga 1140  
tgtcatctta gttaactctgt taccagtacc ataggccaga tactatctag agtcttataa 1200  
acatctatc taatccttct aataaataag cccaagctag gtttttatcc cccattttat 1260  
ggatggggga actgaggcca ataaactcat ataaactatc caaggccaca aaactagttaa 1320  
taaacagagt gaaattccca ccaaaaaaaa actacaatac caaattttct tactcttctc 1380  
ctgtctgtac ttgctgttac tagcaaatgt ctcttggttg gagtattccc atcccctctc 1440  
caaaaaaaaa aaaaaaaaaa gcgrrcac 1468

<210> 59  
<211> 1614  
<212> DNA  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (28)  
<223> n equals a,t,g, or c

<400> 59  
ggaaggatcg atcttattta actatgtntg gaacaaccca gtgaatatca gactcggaat 60  
tactatttca ttctatttca aatgcttata aagctactat tgtagattat agtggttaatg 120  
caaaagttac agacttttga tatggaaac cagataaaaac aatgtttacaa aaggcaaaaat 180  
taaaaggtaw gttttctttt tagtgctttg gaaaaatttc acttaaacctc ttattactgt 240  
atagatttag cctataaatg ctatttatat tccaggggaa cgaaaaatcg aatttgtttt 300  
atgattttaa gcatctggtt tgcataattg attgttaaac tgatacagtt ttgctgtgtc 360  
ccacacaaat tgaattgtgt taatagtctc cataatccct acgtgttgtg ggagggaacc 420  
agtgggcagt aatttaatac tgggtgttgt taccctcatg ctgtctctgt gatgtgagt 480  
ttctcatgaga ctgtaggggt gttttttttt gttttgtttt ttgttttttt agatggagtt 540  
ttgcttttga tgcgccagct ggagtgcaat ggcacacgat ctcggtctac gcgaacctgt 600  
gcctctctggg ttcaagcgat ttctctgctc cagcatctcg agtagctggg attacaggca 660  
tgacacacca cgccacagcta attttgtatt tttagtagag acggggtttc tccatgtttg 720  
ttargctggc ctcaaacctc cgacctcagg tgatccggcc gctctgggct cccaaagtgc 780  
tggtgattaca ggcgtgrcca ctgctcctg cccaagatct gatggttttg taagggaatt 840  
ttcccctttt gcttggcact ttctctgct gccatgtgaa gaaggatgtg ttgtcttccc 900  
ttccaycatg atttgaagt ycatgaggcc tccycagcct gtgggactgt ttgtcttaca 960  
aacgtgttta ctttataaat taccagctct caggcaatc ttatatagca tgtgagaaca 1020  
gactaatatg aaatacaata ctgaaaaaat gttttcttgc tcacctgttc ctatgaacag 1080

```

gaattaaatt ttaaagtatt gccttaagat ggctgtgcta aataataatc attgcaagag 1140
caatactttt acctgtttct agatgacaat attactaaaa tttctcaaat gaagactttg 1200
tttttagcttc aattacttca gaaaatataa attttaaaga tgactatgag ataaatcatg 1260
aactcagtgg aattttcaga tgagatgggg cgcgttcagg gtggtatgac tgtagacgga 1320
attttcagat ctttgtyatt tagaagcaag tatagggtata acgtggacta tcaactgata 1380
tctgcaataa atttgggttaa aaagaaattt gattgtagta tttgttgctg taggattata 1440
aatgtcaaat atcattgttaa acatttctat atttttagaa atatcttggg tggcctgaaa 1500
cagaagtgaag gaaatcaatt ttttaagggtg agccatttgg cttttttaaa aaattgagat 1560
tcaacttaca taccataaag ttcactcttc taaaaaaaaa aaaaaaaaaa tcga 1614

```

&lt;210&gt; 60

&lt;211&gt; 48

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (48)

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 60

```

Met Ile Phe Pro Lys Ile Cys Pro Ile Ser Pro Asn Leu Val Ser Val
 1 5 10 15

```

```

Leu Ser Leu Val Phe Phe Trp Thr Leu Leu Gly Ser Arg Arg Val Cys
 20 25 30

```

```

Tyr Gln Phe Ser Arg Leu Gly Glu Val Arg Ser Gly Glu Gln Leu Xaa
 35 40 45

```

&lt;210&gt; 61

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (41)

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 61

```

Met Lys Asn Pro Ile Thr Lys Arg Trp Lys His Leu Thr Gly Thr Leu
 1 5 10 15

```

```

Ile Leu Val Asn Ser Leu Asp Val Leu Arg Ala Ala Ala Phe Ser Pro
 20 25 30

```

```

Ala Asp Gln Asp Asp Phe Val Ile Xaa
 35 40

```

&lt;210&gt; 62

&lt;211&gt; 33

<212> PRT  
 <213> Homo sapiens  
  
 <220>  
 <221> SITE  
 <222> (33)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids  
  
 <400> 62  
 Met Lys Val Leu Ser Phe Leu Leu Cys Ile Arg Ile Ser Phe Leu Phe  
           1                          5                          10                          15  
  
 Val Val Glu Ser Ile Val Arg Gly Ile Ser Lys Leu Asn Glu Val Asn  
                           20                          25                          30  
  
 Xaa

<210> 63  
 <211> 23  
 <212> PRT  
 <213> Homo sapiens  
  
 <220>  
 <221> SITE  
 <222> (23)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids  
  
 <400> 63  
 Met Val Pro Ser Leu Asn Leu Tyr Leu Leu Val Ser Trp Asp Thr Leu  
           1                          5                          10                          15  
  
 Leu Glu Asn Phe Leu Met Xaa  
                           20

<210> 64  
 <211> 37  
 <212> PRT  
 <213> Homo sapiens  
  
 <220>  
 <221> SITE  
 <222> (37)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids  
  
 <400> 64  
 Met Lys Tyr Ser Cys Leu Lys Gly Ser Tyr Thr Val Phe Met Ala Ile  
           1                          5                          10                          15  
  
 Leu Ala Leu Leu Leu Val Val Cys Ile Phe Tyr Gln Arg Lys Pro Val  
                           20                          25                          30  
  
 Phe Arg Asp Gly Xaa  
                           35

<210> 65  
 <211> 40  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (40)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 65  
 Met Cys Thr Leu Leu Ile Phe Phe Leu Ile Leu Pro His Trp Trp Leu  
           1                  5                  10                  15

Gly Thr Trp Glu Arg Glu Gln Ala Phe Leu His Gly Pro Ser Leu Gly  
                   20                  25                  30

Cys Gly Glu Gln Arg Pro Met Xaa  
           35                  40

<210> 66  
 <211> 27  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (27)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 66  
 Met Thr Val Val Leu Leu Leu His Gly Ile Thr Val Tyr Tyr Val  
           1                  5                  10                  15

Val Gly Lys Tyr Ser Leu Leu Asn Pro Thr Xaa  
                   20                  25

<210> 67  
 <211> 46  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (46)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 67  
 Met Arg Trp Thr Cys Leu Leu Gly Thr Pro Gly His Pro Leu Phe Phe  
           1                  5                  10                  15

Leu Leu Cys Ala Trp Ser Ile Met Ser Thr Pro Ala Asp Pro Trp Lys  
                   20                  25                  30

Arg Lys Cys Leu Cys Cys Arg Val Leu His Gly His Glu Xaa  
           35                  40                  45

<210> 68  
 <211> 37  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (37)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 68  
 Met Leu Tyr Leu Asn Met Asn Gly Lys Phe Trp Phe Leu Ala Phe Thr  
 1 5 10 15

Phe Tyr Tyr Leu Asn Phe Ile Asn Ala Asn Ile Ser Phe Val Ile Ser  
 20 25 30

Tyr Ser Ile Ser Xaa  
 35

<210> 69  
 <211> 25  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (25)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 69  
 Met Gly Gly Thr Pro Ser Thr Lys Cys Leu Val Thr Ser Ala Trp Ser  
 1 5 10 15

Gly Phe Ser Ala Cys Thr Pro Cys Xaa  
 20 25

<210> 70  
 <211> 62  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (62)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 70  
 Met Asn Tyr Arg Cys Leu Thr Phe Lys Ile Tyr Phe Val Leu Leu Ile  
 1 5 10 15

Ser Ser His Cys Val Asn Thr Gly Ser Cys Arg Ser Leu Leu Gly Arg  
 20 25 30



Asn Lys Phe Ile Tyr Phe Leu Arg Gly Ser Ser Gly Val Leu Ile Arg  
 35 40 45

Pro Leu Leu Cys Leu Gly Gly Ala Leu Val Leu Leu Ala Xaa  
 50 55 60

<210> 71

<211> 11

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (11)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 71

Met Gly Gly Val Ser Gly Phe Ser Val Ala Xaa  
 1 5 10

<210> 72

<211> 71

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (71)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 72

Met Ser Pro Ser Gln Ser Gly Val Gly Ile Ser Gly Leu Gly Leu Phe  
 1 5 10 15

Leu Ser Lys Thr Pro Leu Phe Ser Leu Ile Leu Lys Val Ile Phe Leu  
 20 25 30

Arg Thr Glu Leu Leu Pro Lys Glu Arg Asp Gly Phe Pro Arg Arg His  
 35 40 45

Ser Trp Pro Ser Val Asp Leu Ser Val His Leu Leu Ala Leu Leu Ala  
 50 55 60

Asp Ser Ser Gly Trp Ala Xaa  
 65 70

<210> 73

<211> 62

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (62)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 73

Met Cys Leu Leu Leu Trp Leu Thr Thr Phe Gln Arg Thr Ser Gly  
 1 5 10 15

Ala Leu Arg Arg Gly Gly Leu Ser Ser Pro Ala Trp Ala Met Arg Ser  
 20 25 30

Pro Ser Val Tyr Ser Thr Gln Thr Pro Ser Pro Met Met Ser Thr Gly  
 35 40 45

Thr Leu Arg Gly Leu Ser Gly Ala Met Cys Asn Leu Ser Xaa  
 50 55 60

&lt;210&gt; 74

&lt;211&gt; 184

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (184)

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 74

Met Thr Lys Ser Ser Ala Val Leu Phe Ile Leu Ile Phe Ser Leu Ile  
 1 5 10 15

Phe Lys Leu Glu Glu Leu Arg Ala Ala Leu Val Leu Val Val Leu Leu  
 20 25 30

Ile Ala Gly Gly Leu Phe Met Phe Thr Tyr Lys Ser Thr Gln Phe Asn  
 35 40 45

Val Glu Gly Phe Ala Trp Cys Trp Gly Pro Arg Ser Ser Val Ala Phe  
 50 55 60

Ala Gly Pro Ser Pro Arg Cys Ser Cys Arg Arg Leu Asn Ser Ala Ser  
 65 70 75 80

Arg Ile Pro Ser Thr Pro Cys Ser Thr Cys Ser His Ser Cys Ser Trp  
 85 90 95

Gly Ser Ser Leu Ser Leu Leu Tyr Leu Lys Val Ser Ile Cys Pro His  
 100 105 110

Leu Arg Lys Ser Ser Val Ser Arg Thr Gln Gly Cys Ser Cys Gly Tyr  
 115 120 125

Leu Gly Ala Ser Ser Leu Ala Gly Phe Ser Pro Leu Val Trp Ala Ser  
 130 135 140

Leu Ser Ser Ser Trp Ser Pro Glu Pro Pro Ala Ser Leu Ser Pro Leu  
 145 150 155 160

Pro Ala Phe Leu Arg Lys Ser Ala Leu Cys Cys Trp Gln Leu Ile Cys  
 165 170 175

Trp Ala Ile Arg Ser Ala Ser Xaa  
180

<210> 75

<211> 23

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (23)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 75

Met Val Cys Cys Trp Arg Gly Cys Cys Trp Ser Trp Ser Ile Val Leu  
1 5 10 15

Leu Trp Leu Gly Thr Val Xaa  
20

<210> 76

<211> 179

<212> PRT

<213> Homo sapiens

<400> 76

Met Gly Leu Ile Phe Ala Lys Leu Trp Ser Leu Phe Cys Asn Gln Glu  
1 5 10 15

His Lys Val Ile Ile Val Gly Leu Asp Asn Ala Gly Lys Thr Thr Ile  
20 25 30

Leu Tyr Gln Phe Leu Met Asn Glu Val Val His Thr Ser Pro Thr Ile  
35 40 45

Gly Ser Asn Val Glu Glu Ile Val Val Lys Asn Thr His Phe Leu Met  
50 55 60

Trp Asp Ile Gly Gly Gln Glu Ser Leu Arg Ser Ser Trp Asn Thr Tyr  
65 70 75 80

Tyr Ser Asn Thr Glu Phe Ile Ile Leu Val Val Asp Ser Ile Asp Arg  
85 90 95

Glu Arg Leu Ala Ile Thr Lys Glu Glu Leu Tyr Arg Met Leu Ala His  
100 105 110

Glu Asp Leu Arg Lys Ala Ala Val Leu Ile Phe Ala Asn Lys Gln Asp  
115 120 125

Met Lys Gly Cys Met Thr Ala Ala Glu Ile Ser Lys Tyr Leu Thr Leu  
130 135 140

Ser Ser Ile Lys Asp His Pro Trp His Ile Gln Ser Cys Cys Ala Leu  
145 150 155 160

Thr Gly Glu Gly Leu Cys Gln Gly Leu Glu Trp Met Thr Ser Arg Ile  
 165 170 175

Gly Val Arg

<210> 77  
 <211> 39  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (14)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (34)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (39)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 77  
 Met Arg Ser Pro Leu Ser Ala Ser Ala Leu Leu Leu Trp Xaa Ser Ser  
 1 5 10 15

Val Ala Arg Ala Phe Ser Ser Pro Ser Leu Leu Met Ala Val Pro Ser  
 20 25 30

Pro Xaa Cys Ser Arg Tyr Xaa  
 35

<210> 78  
 <211> 66  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (66)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 78  
 Met Asn Leu Ser Lys Ala Pro Ala Leu Arg Phe Leu Trp Ser Cys Ser  
 1 5 10 15

Ser Ile Thr Gly Ala Ala Gly Asn Leu Asn Thr Thr Ser Trp Ser Thr  
 20 25 30

Arg Leu Trp Pro Asn Gly Arg Arg Lys Lys Leu Ser Ser Gly Trp Ser  
 35 40 45

Ser Trp Ala Leu Gly His Leu Phe Thr Gly Lys Gly Phe Tyr Leu Asn  
 50 55 60

Glu Xaa  
 65

<210> 79  
 <211> 48  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (48)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 79  
 Met Val Tyr Arg Ile Gln His Ala Asp Thr Trp Trp Thr Tyr Cys Tyr  
 1 5 10 15

Trp Gly Phe Val Ile Leu Gly Phe Gln Trp Ser Val Leu Val Phe Met  
 20 25 30

Tyr Val Asn Pro Arg Cys Ala Leu Asp Ser Gly Tyr Phe Lys Phe Xaa  
 35 40 45

<210> 80  
 <211> 218  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (156)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (196)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (211)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (218)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 80

Met Leu Pro Val Val Trp Ile Ile Leu Cys Ser Ser Ala Gln Phe Pro  
 1 5 10 15

His Leu Trp Lys Leu His Ser His Leu Ala Gln Met Ser His Ser Ser  
 20 25 30

Leu Leu Pro Leu Glu Asn Arg Ser Leu Ser Leu Cys Arg Thr Arg Cys  
 35 40 45

Ser Asp Pro Leu Ser Cys Thr Leu Ala Pro Phe Leu Pro Pro Thr Val  
 50 55 60

Ser Leu Ser Thr Leu Ser Ser Ala Leu Gln Ser Ser Phe Cys Ser Phe  
 65 70 75 80

Phe Leu Arg Arg Gln Cys Arg Pro Ser Cys Ser Pro Val Pro Gly Val  
 85 90 95

His Ile Ser Asn Phe Gly Ser Ala Ser Trp Thr Cys Pro Cys Gly Cys  
 100 105 110

Leu Ala Ile His Ser Asn Pro Ala Ser Pro Lys Gly Met Ile Leu Pro  
 115 120 125

Tyr Phe Leu Leu Thr Gln Leu Cys Gly Gln Ser Ser Arg Thr Asn Gly  
 130 135 140

Ser Lys Leu Pro Pro Asn Thr His Pro Arg Leu Xaa Ala Trp Ala Pro  
 145 150 155 160

Leu Ala Ser Pro Arg Ala Ala His Ile Lys Val Gln Leu Gly Ser Glu  
 165 170 175

Leu Leu Gln Glu Ala Ser Pro Ala Leu Leu Cys Arg Arg Thr Leu Leu  
 180 185 190

Leu His Thr Xaa Ser Pro Ile Leu Trp Gln Ala Leu Leu Tyr Pro Gln  
 195 200 205

Ala Ser Xaa Gln Thr Ser Leu Thr Cys Xaa  
 210 215

<210> 81

<211> 57

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (57)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 81

Met Lys Ser His Ser Val Trp Leu Pro Thr Leu Tyr Cys Ala Val Val  
 1 5 10 15

Lys Val Tyr Leu Cys Val Gly Cys Ser His Pro Leu Val Ser Thr Gly

55

```

 20 25 30
Ile Gly Pro Arg Ser Leu His Arg Ser Pro Ala Gly Met Pro Val Ser
 35 40 45

His Ser Ala Pro Cys Lys Thr His Xaa
 50 55

<210> 82
<211> 38
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (38)
<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 82
Met Lys Pro Met Pro Leu Ser Glu Gly Lys Ser Ile Leu Leu Phe Gly
 1 5 10 15

Gly Ala Ala Ala Val Ala Ile Leu Ala Val Ala Ile Gly Val Ala Leu
 20 25 30

Ala Leu Arg Lys Lys Xaa
 35

<210> 83
<211> 29
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (29)
<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 83
Met Pro Val Trp Lys Cys Leu Leu Trp Cys Thr Pro Leu Leu Arg Cys
 1 5 10 15

Thr Gln Leu Leu Leu Gln Leu Gln Ser Arg Cys Cys Xaa
 20 25

<210> 84
<211> 29
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (29)
<223> Xaa equals any of the twenty naturally occurring L-amino acids

```

&lt;400&gt; 84

Met Val Phe Gln Thr Phe Tyr Cys Ile Ser Leu Val Leu Phe Ala His  
1 5 10 15

Glu Val Ser Leu Leu Cys Val Cys Val Tyr Val Tyr Xaa  
20 25

&lt;210&gt; 85

&lt;211&gt; 19

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (19)

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 85

Met Gln Lys Val Thr Thr Leu Arg His Ile Glu Asn Cys Leu Ala Gly  
1 5 10 15

Lys Cys Xaa

&lt;210&gt; 86

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (25)

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 86

Met Val Val Leu Leu Glu Ile Phe Leu Leu Leu Ala Tyr Val Leu Trp  
1 5 10 15

Asn Tyr Thr Cys Ile Thr Asn Asp Xaa  
20 25

&lt;210&gt; 87

&lt;211&gt; 17

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (17)

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 87

Phe Leu Ile Ser Leu Cys Ile Leu Ile Pro Phe Leu Ile Leu Ser Pro  
1 5 10 15



Xaa

<210> 88  
 <211> 98  
 <212> PRT  
 <213> Homo sapiens  
  
 <220>  
 <221> SITE  
 <222> (98)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 88  
 Glu Phe Gly Phe Phe Ser Leu Phe Leu Val Ile Ser Leu Phe Val  
 1 5 10 15  
 Trp Lys Glu Ser Tyr Phe Ser Leu Asp Ile Thr Pro Glu Phe Pro Ser  
 20 25 30  
 Asp Ala Leu Leu Arg Val Arg Ala Val Ala Asp Ser Leu Lys Phe Ser  
 35 40 45  
 Ser Ala Leu Pro Tyr Leu Pro Phe Ile Leu Gly Ile Gly Lys Cys Cys  
 50 55 60  
 Lys Gly Pro Asp Gly Lys Tyr Phe Arg Leu Tyr Gly Leu Val Ser Val  
 65 70 75 80  
 Ala Asn Ile Gln Leu Cys Gln Cys Thr Leu Lys Ala Ala Ile Asp Asn  
 85 90 95

Met Xaa

<210> 89  
 <211> 43  
 <212> PRT  
 <213> Homo sapiens  
  
 <220>  
 <221> SITE  
 <222> (43)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 89  
 Met Asn Cys Gly Thr Arg Gly Cys Glu Asn Leu Arg Trp Asn Leu Ser  
 1 5 10 15  
 Phe Phe Leu Ser Phe Phe Leu Asn Phe Val Phe Ser Ile Gln Gln Gln  
 20 25 30  
 Cys Val Glu Arg Ile Leu Cys Arg Cys Arg Xaa  
 35 40

<210> 90  
 <211> 3  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (3)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 90  
 Phe Pro Xaa  
 1

<210> 91  
 <211> 51  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (51)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 91  
 Met Leu Pro Pro Ser Leu Leu Phe Ile Phe Ser Ala Phe Cys Leu  
 1 5 10 15

Ala Ser Leu Pro Pro Cys Phe Ser Gly Trp Glu Leu Leu Val Thr Pro  
 20 25 30

Leu Ser Asp Cys Leu Val Ala Gly Thr Leu Pro Val Arg Ala Thr Phe  
 35 40 45

Leu Phe Xaa  
 50

<210> 92  
 <211> 5  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (5)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 92  
 Tyr Ser Leu Thr Xaa  
 1 5

<210> 93  
 <211> 37  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (37)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 93  
 Met Glu Lys Arg Glu Ala Tyr Ile Ser Ser His Leu Leu Ser Met Ser  
 1 5 10 15  
 Leu Leu Arg Thr Cys Leu Ile Leu Arg Met Val Val Trp Leu Ser Ser  
 20 25 30  
 Leu Gly Arg Arg Xaa  
 35

<210> 94  
 <211> 131  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (131)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 94  
 Met Tyr Gln Thr Pro Met Glu Val Ala Val Tyr Gln Leu His Asn Phe  
 1 5 10 15  
 Ser Ile Ser Phe Phe Ser Ser Leu Leu Gly Gly Asp Val Val Ser Val  
 20 25 30  
 Lys Leu Asp Asn Ser Ala Ser Gly Ala Ser Val Val Ala Ile Asp Asn  
 35 40 45  
 Lys Ile Glu Gln Ala Met Asp Leu Val Lys Asn His Leu Met Tyr Ala  
 50 55 60  
 Val Arg Glu Glu Val Glu Ile Leu Lys Glu Gln Ile Arg Glu Leu Val  
 65 70 75 80  
 Glu Lys Asn Ser Gln Leu Glu Arg Glu Asn Thr Leu Leu Lys Thr Leu  
 85 90 95  
 Ala Ser Pro Glu Gln Leu Glu Lys Phe Gln Ser Cys Leu Ser Pro Glu  
 100 105 110  
 Glu Pro Ala Pro Glu Ser Pro Gln Val Pro Glu Ala Pro Gly Gly Ser  
 115 120 125  
 Ala Val Xaa  
 130

<210> 95  
 <211> 71

60

```

<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (71)
<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 95
Met Cys Asp Val Leu Trp Gly Leu Phe Ile Leu Leu Val His Arg Arg
 1 5 10 15

Gly Thr Leu Pro Gln Glu Ser Arg His Leu Ser Ser Ser Ser Ser Ser
 20 25 30

Ser Ser Ala Thr Glu Glu Pro Pro Tyr Leu Gly Gln Val Ser His Cys
 35 40 45

Leu Pro Val Met Phe Thr Thr His Glu Arg Thr Arg Arg Phe Asp Val
 50 55 60

Ser Ile Lys Leu Asn Glu Xaa
 65 70

<210> 96
<211> 25
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (25)
<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 96
Ile Arg Arg Val Leu Trp Ala Pro Gly Gln Pro Ser Cys Leu Ser Ser
 1 5 10 15

Pro Ala Val Ser Arg Gly Arg Trp Xaa
 20 25

<210> 97
<211> 18
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (18)
<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 97
Met Trp Leu Ser Arg Glu Val Leu Val Leu Ile Ser Ile Ser Ala Ser
 1 5 10 15

Gly Xaa

```

<210> 98  
 <211> 24  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (24)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 98  
 Met Phe Gln Ala Pro Ala Leu Ala Phe Leu Ser Gly Leu Gln Leu Ser  
 1 5 10 15

Phe Val Ala Cys Ser Ile Tyr Xaa  
 20

<210> 99  
 <211> 52  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (52)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 99  
 Met Thr Leu Leu Trp Lys Phe Asn Leu Phe Ile Val Phe Trp Ala  
 1 5 10 15

Leu Trp Val Val Arg Ala Gly Leu Trp Val Leu Gly Asn Ser Ser Gly  
 20 25 30

Ser Gln His Ser Lys Ala Lys Leu Thr Ser Ser Ser His Thr Leu Gln  
 35 40 45

Glu Lys His Xaa  
 50

<210> 100  
 <211> 25  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (25)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 100  
 Met Cys Trp Pro Trp Gly Gly Pro Pro Leu Pro Ser Leu Pro Thr Ser  
 1 5 10 15

Leu Arg Trp Met Ser Leu Cys Tyr Xaa  
 20 25

<210> 101

<211> 130

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (130)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 101

Met Val Asp Leu Ile Gly Glu Val Leu Leu Pro Leu Leu Gly Gln Glu  
 1 5 10 15

Ala Glu Ala Cys Thr Ala Asp Asp Pro Ala Asp His Val Lys Val Pro  
 20 25 30

Ala His Ala Ala Val His Val Val Gln Asn His Ala Leu Leu Gly His  
 35 40 45

Val Val Phe Asp Asp Asp Ala Val Gly Ala Glu Ala Ala Leu Ala  
 50 55 60

Ala Pro Gln Glu Leu Gly Gln Val Leu Ile Gly Glu Val Ala Trp Gly  
 65 70 75 80

Gly Gln Arg Gln Gly Tyr Gln Gly Thr Met Gln Ala Asp Val Gly Thr  
 85 90 95

Gly Trp Asp Val Leu Leu Ser Pro Ala Glu Pro Leu Ile Cys Ala Gln  
 100 105 110

His Phe Val Cys Thr His Gly Leu Ile Gly Ser Gln Ser Arg Glu Ile  
 115 120 125

Tyr Xaa

130

<210> 102

<211> 33

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (33)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 102

Met Ser Leu Leu Ile Asn Arg Met Trp Pro Trp Val Ser Thr Glu  
 1 5 10 15

Pro Leu Leu Ala Ser Ala Ser Ser Phe Met Trp Glu Phe Ser Ser Thr  
                   20                                  25                                  30

Xaa

<210> 103  
 <211> 10  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (10)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 103  
 Met Val Leu Ser Pro Leu Phe Ser Ala Xaa  
           1                                  5                                  10

<210> 104  
 <211> 224  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (137)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 104  
 Met Glu Ser Gly Ala Tyr Gly Ala Ala Lys Ala Gly Gly Ser Phe Asp  
           1                                  5                                  10                                  15

Leu Arg Arg Phe Leu Thr Gln Pro Gln Val Val Ala Arg Ala Val Cys  
                   20                                  25                                  30

Leu Val Phe Ala Leu Ile Val Phe Ser Cys Ile Tyr Gly Glu Gly Tyr  
                   35                                  40                                  45

Ser Asn Ala His Glu Ser Lys Gln Met Tyr Cys Val Phe Asn Arg Asn  
                   50                                  55                                  60

Glu Asp Ala Cys Arg Tyr Gly Ser Ala Ile Gly Val Leu Ala Phe Leu  
           65                                  70                                  75                                  80

Ala Ser Ala Phe Phe Leu Val Val Asp Ala Tyr Phe Pro Gln Ile Ser  
                   85                                  90                                  95

Asn Ala Thr Asp Arg Lys Tyr Leu Val Ile Gly Asp Leu Leu Phe Ser  
                   100                                  105                                  110

Ala Leu Trp Thr Phe Leu Trp Phe Val Gly Phe Cys Phe Leu Thr Asn  
           115                                  120                                  125

Gln Trp Ala Val Thr Asn Pro Lys Xaa Val Leu Val Gly Ala Asp Ser

130                      135                      140  
 Val Arg Ala Ala Ile Thr Phe Ser Phe Phe Ser Ile Phe Ser Trp Gly  
 145                      150                      155                      160  
 Val Leu Ala Ser Leu Ala Tyr Gln Arg Tyr Lys Ala Gly Val Asp Asp  
                     165                      170                      175  
 Phe Ile Gln Asn Tyr Val Asp Pro Thr Pro Asp Pro Asn Thr Ala Tyr  
                     180                      185                      190  
 Ala Ser Tyr Pro Gly Ala Ser Val Asp Asn Tyr Gln Gln Pro Pro Phe  
                     195                      200                      205  
 Thr Gln Asn Ala Glu Thr Thr Glu Gly Tyr Gln Pro Pro Val Tyr  
                     210                      215                      220

<210> 105  
 <211> 14  
 <212> PRT  
 <213> Homo sapiens  
  
 <220>  
 <221> SITE  
 <222> (14)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 105  
 Met Glu Gly Thr Gly Ser Gly Asn Gly Arg Tyr Cys Met Xaa  
   1                      5                      10

<210> 106  
 <211> 52  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (43)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (52)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 106  
 Met Arg Ala Phe Ile Trp Thr Cys Gly Thr Trp Thr Leu Trp Ser Leu  
   1                      5                      10                      15  
 Ser Asp Arg Ser Leu Gly Ser Ser Arg Ile Cys Met Gly Phe Ala Gly  
                     20                      25                      30



Ser Leu Lys Arg Pro Gly Arg Thr Lys Ala Xaa Thr Thr Phe Trp Gly  
 35 40 45

Thr Trp Phe Xaa  
 50

<210> 107

<211> 45

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (45)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 107

Met Asn Asn Thr Cys Leu Ala Val Leu His Val Val Met Cys Leu Ala  
 1 5 10 15

Trp Ser Phe Leu Trp Ser Leu Leu Leu His Gly Phe Ile His Ser Phe  
 20 25 30

Phe Ser Phe Ile Ser Ser Cys Ala Val Thr Cys Thr Xaa  
 35 40 45

<210> 108

<211> 11

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (11)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 108

Met Val Val Val Thr Leu Met Leu Phe Leu Xaa  
 1 5 10

<210> 109

<211> 72

<212> PRT

<213> Homo sapiens

<400> 109

Leu Arg Gln Ser Leu Asn Ser Val Thr Gln Ala Gly Val Gln Trp Arg  
 1 5 10 15

Asn Leu Gly Ser Leu Gln Pro Leu Pro Pro Gly Phe Lys Leu Phe Ser  
 20 25 30

Cys Pro Ser Leu Leu Ser Ser Trp Asp Tyr Arg Arg Pro Pro Arg Leu  
 35 40 45

Ala Asn Phe Phe Val Phe Leu Val Glu Met Gly Phe Thr Met Phe Ala  
 50 55 60

Arg Leu Ile Leu Ile Ser Gly Pro  
 65 70

<210> 110  
 <211> 73  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (56)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (72)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 110  
 Leu Arg Cys Ser Phe Ala Leu Val Ala Gln Ala Gly Val Gln Trp Cys  
 1 5 10 15

Asp Leu Gly Ser Leu Gln Pro Pro Pro Lys Phe Lys Pro Phe Cys  
 20 25 30

Cys Pro Arg Leu Leu Ser Gly Trp Asp Tyr Arg His Pro Pro Pro Cys  
 35 40 45

Leu Thr Asn Phe Leu Tyr Phe Xaa Lys Arg Trp Gly Phe Thr Met Leu  
 50 55 60

Ala Arg Leu Leu Leu Asn Ser Xaa Pro  
 65 70

<210> 111  
 <211> 38  
 <212> PRT  
 <213> Homo sapiens

<400> 111  
 Asn Pro Ile Thr Lys Arg Trp Lys His Leu Thr Gly Thr Leu Ile Leu  
 1 5 10 15

Val Asn Ser Leu Asp Val Leu Arg Ala Ala Ala Phe Ser Pro Ala Asp  
 20 25 30

Gln Asp Asp Phe Val Ile  
 35

<210> 112  
 <211> 38  
 <212> PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 112

Asn Pro Ile Thr Lys Arg Trp Lys His Leu Thr Gly Thr Leu Ile Leu  
 1 5 10 15  
 Val Asn Ser Leu Asp Val Leu Arg Ala Ala Phe Ser Pro Ala Asp  
 20 25 30  
 Gln Asp Asp Phe Val Ile  
 35

&lt;210&gt; 113

&lt;211&gt; 294

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 113

Leu Ile Thr Asp Leu Asp His Asp Ser Lys Asp Gly Lys Lys Trp Lys  
 1 5 10 15  
 Ser Leu Val Ser Arg Gly Phe Leu Lys Val Ser Ala Asp His Lys His  
 20 25 30  
 Ala Asp Ile His Phe Asp Lys Asp Ser Glu Tyr Tyr Val Asp Thr Asn  
 35 40 45  
 Ile Ala Ala Gly Gly Arg Ala Met Glu Leu Ser Asp Leu Ala Val Phe  
 50 55 60  
 Asn Gly Lys Leu Tyr Ser Ile Asp Asp Arg Thr Gly Leu Ile Tyr Gln  
 65 70 75 80  
 Ile Ser Asp Lys Lys Ala Leu Pro Trp Val Leu Leu Asn Asp Gly Pro  
 85 90 95  
 Gly Asn Val Val Lys Gly Phe Lys Gly Glu Trp Ile Thr Val Lys Asp  
 100 105 110  
 Thr Glu Leu Ile Val Gly Gly Leu Gly Lys Glu Trp Thr Thr Thr Asp  
 115 120 125  
 Gly Val Tyr Val Asn Asp His Pro Met Trp Val Lys His Val Ser Ala  
 130 135 140  
 His Gly Ala Val His His Glu Asn Trp Lys Asp Val Tyr Ile Arg Val  
 145 150 155 160  
 Arg Arg Ala Ala Gly Ile Glu Tyr Pro Gly Tyr Met Ile His Glu Ala  
 165 170 175  
 Val Gln Trp Ser Ala Ile His Arg Lys Trp Phe Phe Leu Pro Arg Arg  
 180 185 190  
 Met Ser Asn Glu Lys Tyr Ser Glu Ala Glu Asp Glu Asn Arg Gly Thr  
 195 200 205

Asn Val Leu Val Ile Gly Asn Glu Glu Leu Thr Asp Phe Glu Val Val  
 210 215 220  
 Arg Val Gly Ser Glu Asn Asn Lys Ser Arg Gly Phe Ala Ala Phe Gln  
 225 230 235 240  
 Phe Val Pro Asn Thr His His Gln Leu Ile Val Ala Ile Lys Ser Glu  
 245 250 255  
 Glu Lys Asp Gly Lys Pro Val Ala Ser Tyr Ala Ser Val Phe Asp Ile  
 260 265 270  
 His Gly Asn Val Ile Leu Asp Glu Tyr Leu Leu His Gly Pro Tyr Lys  
 275 280 285  
 Tyr Glu Gly Ile Ala Phe  
 290

<210> 114  
 <211> 292  
 <212> PRT  
 <213> Homo sapiens

<400> 114  
 Val Ile Ala Asp Leu Asp Thr Glu Ser Arg Ala Gln Glu Glu Asn Thr  
 1 5 10 15  
 Trp Phe Ser Tyr Leu Lys Lys Gly Tyr Leu Thr Leu Ser Asp Ser Gly  
 20 25 30  
 Asp Lys Val Ala Val Glu Trp Asp Lys Asp His Gly Val Leu Glu Ser  
 35 40 45  
 His Leu Ala Glu Lys Gly Arg Gly Met Glu Leu Ser Asp Leu Ile Val  
 50 55 60  
 Phe Asn Gly Lys Leu Tyr Ser Val Asp Asp Arg Thr Gly Val Val Tyr  
 65 70 75 80  
 Gln Ile Glu Gly Ser Lys Ala Val Pro Trp Val Ile Leu Ser Asp Gly  
 85 90 95  
 Asp Gly Thr Val Glu Lys Gly Phe Lys Ala Glu Trp Leu Ala Val Lys  
 100 105 110  
 Asp Glu Arg Leu Tyr Val Gly Gly Leu Gly Lys Glu Trp Thr Thr Thr  
 115 120 125  
 Thr Gly Asp Val Val Asn Glu Asn Pro Glu Trp Val Lys Val Val Gly  
 130 135 140  
 Tyr Lys Gly Ser Val Asp His Glu Asn Trp Val Ser Asn Tyr Asn Ala  
 145 150 155 160  
 Leu Arg Ala Ala Ala Gly Ile Gln Pro Pro Gly Tyr Leu Ile His Glu  
 165 170 175

Ser Ala Cys Trp Ser Asp Thr Leu Gln Arg Trp Phe Phe Leu Pro Arg  
 180 185 190

Arg Ala Ser Gln Glu Arg Tyr Ser Glu Lys Asp Asp Glu Arg Lys Gly  
 195 200 205

Ala Asn Leu Leu Leu Ser Ala Ser Pro Asp Phe Gly Asp Ile Ala Val  
 210 215 220

Ser His Val Gly Ala Val Val Pro Thr His Gly Phe Ser Ser Phe Lys  
 225 230 235 240

Phe Ile Pro Asn Thr Asp Asp Gln Ile Ile Val Ala Leu Lys Ser Glu  
 245 250 255

Glu Asp Ser Gly Arg Val Ala Ser Tyr Ile Met Ala Phe Thr Leu Asp  
 260 265 270

Gly Arg Phe Leu Leu Pro Glu Thr Lys Ile Gly Ser Val Lys Tyr Glu  
 275 280 285

Gly Ile Glu Phe  
 290

&lt;210&gt; 115

&lt;211&gt; 438

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 115

Asp Ala Phe Gly Asn Ser Glu Gly Lys Glu Met Val Ala Ala Gly Asp  
 1 5 10 15

Asn Lys Val Lys Gln Gly Leu Leu Pro Ser Leu Glu Asp Leu Leu Phe  
 20 25 30

Tyr Thr Ile Ala Glu Gly Gln Glu Lys Ile Pro Val His Lys Phe Ile  
 35 40 45

Thr Ala Leu Lys Ser Thr Gly Leu Arg Thr Ser Asp Pro Arg Leu Lys  
 50 55 60

Glu Cys Met Asp Met Leu Arg Leu Thr Leu Gln Thr Thr Ser Asp Gly  
 65 70 75 80

Val Met Leu Asp Lys Asp Leu Phe Lys Lys Cys Val Gln Ser Asn Ile  
 85 90 95

Val Leu Leu Thr Gln Ala Phe Arg Arg Lys Phe Val Ile Pro Asp Phe  
 100 105 110

Met Ser Phe Thr Ser His Ile Asp Glu Leu Tyr Glu Ser Ala Lys Lys  
 115 120 125

Gln Ser Gly Gly Lys Val Ala Asp Tyr Ile Pro Gln Leu Ala Lys Phe  
 130 135 140

Ser Pro Asp Leu Trp Gly Val Ser Val Cys Thr Val Asp Gly Gln Arg  
 145 150 155 160  
 His Ser Ile Gly Asp Thr Lys Val Pro Phe Cys Leu Gln Ser Cys Val  
 165 170 175  
 Lys Pro Leu Lys Tyr Ala Ile Ala Val Asn Asp Leu Gly Thr Gly Tyr  
 180 185 190  
 Val His Arg Tyr Val Gly Lys Glu Pro Ser Gly Leu Arg Phe Asn Lys  
 195 200 205  
 Leu Phe Leu Asn Glu Asp Asp Lys Pro His Asn Pro Met Val Asn Ala  
 210 215 220  
 Gly Ala Ile Val Val Thr Ser Leu Ile Lys Gln Gly Val Asn Asn Ala  
 225 230 235 240  
 Glu Lys Phe Asp Tyr Val Met Gln Phe Leu Asn Lys Met Ala Gly Asn  
 245 250 255  
 Glu Tyr Val Gly Phe Ser Asn Ala Thr Phe Gln Ser Glu Arg Glu Ser  
 260 265 270  
 Gly Asp Arg Asn Phe Ala Ile Gly Tyr Tyr Leu Lys Glu Lys Lys Cys  
 275 280 285  
 Phe Pro Glu Gly Thr Asp Met Val Gly Ile Leu Asp Phe Tyr Phe Gln  
 290 295 300  
 Leu Cys Ser Ile Glu Val Thr Cys Glu Ser Ala Ser Val Met Ala Ala  
 305 310 315 320  
 Thr Leu Ala Asn Gly Gly Phe Cys Pro Ile Thr Gly Glu Arg Val Leu  
 325 330 335  
 Ser Pro Glu Ala Val Arg Asn Thr Leu Ser Leu Met His Ser Cys Gly  
 340 345 350  
 Met Tyr Asp Phe Ser Gly Gln Phe Ala Phe His Val Gly Leu Pro Ala  
 355 360 365  
 Lys Ser Gly Val Ala Gly Gly Ile Leu Leu Val Val Pro Asn Val Met  
 370 375 380  
 Gly Met Met Cys Trp Ser Pro Pro Leu Asp Lys Met Gly Asn Ser Val  
 385 390 395 400  
 Lys Gly Ile His Phe Cys His Asp Leu Val Ser Leu Cys Asn Phe His  
 405 410 415  
 Asn Tyr Asp Asn Leu Arg His Phe Ala Lys Lys Leu Asp Pro Arg Arg  
 420 425 430  
 Glu Gly Gly Asp Gln Arg  
 435

<210> 116  
 <211> 438  
 <212> PRT  
 <213> Homo sapiens

<400> 116

Asp Ala Phe Gly Asn Ser Glu Gly Lys Glu Leu Val Ala Ser Gly Glu  
 1 5 10 15

Asn Lys Ile Lys Gln Gly Leu Leu Pro Ser Leu Glu Asp Leu Leu Phe  
 20 25 30

Tyr Thr Ile Ala Glu Gly Gln Glu Lys Ile Pro Val His Lys Phe Ile  
 35 40 45

Thr Ala Leu Lys Ser Thr Gly Leu Arg Thr Ser Asp Pro Arg Leu Lys  
 50 55 60

Glu Cys Met Asp Met Leu Arg Leu Thr Leu Gln Thr Thr Ser Asp Gly  
 65 70 75 80

Val Met Leu Asp Lys Asp Leu Phe Lys Lys Cys Val Gln Ser Asn Ile  
 85 90 95

Val Leu Leu Thr Gln Ala Phe Arg Arg Lys Phe Val Ile Pro Asp Phe  
 100 105 110

Met Ser Phe Thr Ser His Ile Asp Glu Leu Tyr Glu Ser Ala Lys Lys  
 115 120 125

Gln Ser Gly Gly Lys Val Ala Asp Tyr Ile Pro Gln Leu Ala Lys Phe  
 130 135 140

Ser Pro Asp Leu Trp Gly Val Ser Val Cys Thr Val Asp Gly Gln Arg  
 145 150 155 160

His Ser Thr Gly Asp Thr Lys Val Pro Phe Cys Leu Gln Ser Cys Val  
 165 170 175

Lys Pro Leu Lys Tyr Ala Ile Ala Val Asn Asp Leu Gly Thr Glu Tyr  
 180 185 190

Val His Arg Tyr Val Gly Lys Glu Pro Ser Gly Leu Arg Phe Asn Lys  
 195 200 205

Leu Phe Leu Asn Glu Asp Asp Lys Pro His Asn Pro Met Val Asn Ala  
 210 215 220

Gly Ala Ile Val Val Thr Ser Leu Ile Lys Gln Gly Val Asn Asn Ala  
 225 230 235 240

Glu Lys Phe Asp Tyr Val Met Gln Phe Leu Asn Lys Met Ala Gly Asn  
 245 250 255

Glu Tyr Val Gly Phe Ser Asn Ala Thr Phe Gln Ser Glu Arg Glu Ser  
 260 265 270

Gly Asp Arg Asn Phe Ala Ile Gly Tyr Tyr Leu Lys Glu Lys Lys Cys

275                      280                      285  
 Phe Pro Glu Gly Thr Asp Met Val Gly Ile Leu Asp Phe Tyr Phe Gln  
     290                      295                      300  
 Leu Cys Ser Ile Glu Val Thr Cys Glu Ser Ala Ser Val Met Ala Ala  
     305                      310                      315                      320  
 Thr Leu Ala Asn Gly Gly Phe Cys Pro Ile Thr Gly Glu Arg Val Leu  
                     325                      330                      335  
 Ser Pro Glu Ala Val Arg Asn Thr Leu Ser Leu Met His Ser Cys Gly  
                     340                      345                      350  
 Met Tyr Asp Phe Ser Gly Gln Phe Ala Phe His Val Gly Leu Pro Ala  
     355                      360                      365  
 Lys Ser Gly Val Ala Gly Gly Ile Leu Leu Val Val Pro Asn Val Met  
     370                      375                      380  
 Gly Met Met Cys Trp Ser Pro Pro Leu Asp Lys Met Gly Asn Ser Val  
     385                      390                      395                      400  
 Lys Gly Ile His Phe Cys His Asp Leu Val Ser Leu Cys Asn Phe His  
                     405                      410                      415  
 Asn Tyr Asp Asn Leu Arg His Phe Ala Lys Lys Leu Asp Pro Arg Arg  
     420                      425                      430  
 Glu Gly Gly Asp Gln Arg  
     435  
  
 <210> 117  
 <211> 466  
 <212> FRT  
 <213> Homo sapiens  
  
 <400> 117  
 Val Asp Glu Gln Asn Val Pro Glu Ile Leu Thr Leu Tyr Asp Gln Ala  
     1                      5                      10                      15  
 Phe Pro Asp Leu Thr Glu Arg Phe Phe Arg Asp Arg Met Trp Pro Asp  
                     20                      25                      30  
 Glu Asn Val Val Glu Arg Ile Ile Gly Pro Gly Asn Lys Leu Phe Ile  
     35                      40                      45  
 Ile Leu Tyr Lys Glu Leu Tyr Tyr Arg Gln Leu Tyr Ala Arg Asn Thr  
     50                      55                      60  
 Arg Gly Pro Leu Leu Val His Arg Tyr Glu Ser Phe Met Asn Tyr Gln  
     65                      70                      75                      80  
 Glu Leu Phe Ser Glu Leu Leu Ser Ser Lys Asp Pro Ile Pro Leu Ser  
                     85                      90                      95  
 Leu Pro Asn Val Trp Leu Trp Asp Ile Ile Asp Glu Phe Val Tyr Gln



| 100 |     |     |     |     |     |     |     |     |     | 105 |     |     |     |     | 110 |  |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|
| Phe | Gln | Ala | Phe | Cys | Leu | Tyr | Lys | Ala | Asn | Pro | Gly | Lys | Arg | Asn | Ala |  |  |  |  |
|     | 115 |     |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |  |  |  |
| Asp | Glu | Val | Glu | Asp | Leu | Ile | Asn | Ile | Glu | Glu | Asn | Gln | Asn | Ala | Trp |  |  |  |  |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |  |  |
| Asn | Ile | Tyr | Pro | Val | Leu | Asn | Ile | Leu | Tyr | Ser | Leu | Leu | Ser | Lys | Ser |  |  |  |  |
|     | 145 |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |  |  |  |
| Gln | Ile | Val | Glu | Gln | Leu | Lys | Ala | Leu | Lys | Glu | Lys | Arg | Asn | Pro | Asp |  |  |  |  |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |  |  |
| Ser | Val | Ala | Asp | Glu | Phe | Gly | Gln | Ser | Asp | Leu | Tyr | Phe | Lys | Leu | Gly |  |  |  |  |
|     |     | 180 |     |     |     |     | 185 |     |     |     |     |     | 190 |     |     |  |  |  |  |
| Tyr | Phe | Ala | Leu | Ile | Gly | Leu | Leu | Arg | Thr | His | Val | Leu | Leu | Gly | Asp |  |  |  |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |  |  |
| Tyr | His | Gln | Ala | Leu | Lys | Thr | Val | Gln | Tyr | Val | Asp | Ile | Asp | Pro | Lys |  |  |  |  |
|     |     | 210 |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |  |  |  |
| Gly | Ile | Tyr | Asn | Thr | Val | Pro | Thr | Cys | Leu | Val | Thr | Leu | His | Tyr | Phe |  |  |  |  |
|     | 225 |     |     | 230 |     |     |     |     | 235 |     |     |     |     |     | 240 |  |  |  |  |
| Val | Gly | Phe | Ser | His | Leu | Met | Met | Arg | Asn | Tyr | Gly | Glu | Ala | Thr | Lys |  |  |  |  |
|     |     |     | 245 |     |     |     |     | 250 |     |     |     |     |     | 255 |     |  |  |  |  |
| Met | Phe | Val | Asn | Cys | Leu | Leu | Tyr | Ile | Gln | Arg | Thr | Lys | Ser | Val | Gln |  |  |  |  |
|     |     | 260 |     |     |     |     | 265 |     |     |     |     |     | 270 |     |     |  |  |  |  |
| Asn | Gln | Gln | Pro | Ser | Lys | Lys | Asn | Phe | Gln | Tyr | Asp | Val | Ile | Gly | Lys |  |  |  |  |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |  |  |
| Thr | Trp | Asp | Gln | Leu | Phe | His | Leu | Leu | Ala | Ile | Cys | Leu | Ala | Ile | Gln |  |  |  |  |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |  |  |
| Pro | Gln | Arg | Ile | Asp | Glu | Ser | Ile | Ala | Ser | Gln | Leu | Ser | Glu | Arg | Cys |  |  |  |  |
|     | 305 |     |     |     | 310 |     |     |     | 315 |     |     |     |     | 320 |     |  |  |  |  |
| Gly | Glu | Arg | Met | Met | His | Met | Ala | Asn | Gly | Asn | Ile | Asp | Glu | Phe | Arg |  |  |  |  |
|     |     |     | 325 |     |     |     |     | 330 |     |     |     |     |     | 335 |     |  |  |  |  |
| Asn | Ala | Phe | Ala | Thr | Gly | Cys | Pro | Lys | Phe | Leu | Ser | Pro | Thr | Thr | Val |  |  |  |  |
|     |     | 340 |     |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |  |  |
| Val | Tyr | Glu | Gly | Val | Asn | Gln | Ser | Lys | Glu | Pro | Leu | Leu | Arg | Gln | Thr |  |  |  |  |
|     |     | 355 |     |     |     | 360 |     |     |     |     |     | 365 |     |     |     |  |  |  |  |
| Gln | Ser | Phe | Leu | Glu | Gly | Ile | Glu | Ser | Gln | Met | Ala | Leu | Pro | Val | Leu |  |  |  |  |
|     |     | 370 |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |  |  |  |
| Arg | Gly | Tyr | Leu | Lys | Leu | Tyr | Thr | Thr | Leu | Pro | Thr | Lys | Lys | Leu | Ala |  |  |  |  |
|     | 385 |     |     |     | 390 |     |     |     | 395 |     |     |     |     | 400 |     |  |  |  |  |
| Ser | Phe | Met | Asp | Val | Asp | Asp | Glu | His | Tyr | Asp | Ser | Phe | Ile | Gly | Lys |  |  |  |  |
|     |     | 405 |     |     |     |     |     | 410 |     |     |     |     | 415 |     |     |  |  |  |  |

Leu Leu Thr Tyr Lys Met Ile Val Asn Glu Leu Gly Lys Glu Ala Gly  
420 425 430

Pro Ser Ser Ala Asp Asp Asp Glu Pro Gln Thr Asp Ile Asp Phe Tyr  
435 440 445

Val Asp Arg Asp Met Ile Asn Ile Ala Asp Thr Lys Val Ala Arg His  
450 455 460

Val Gly  
465

<210> 118

<211> 458

<212> PRT

<213> Homo sapiens

<400> 118

Val Ile Asp Gln Lys Val Tyr Glu Ile Gln Asp Ile Tyr Glu Asn Ser  
1 5 10 15

Trp Thr Lys Leu Thr Glu Arg Phe Phe Lys Asn Thr Pro Trp Pro Glu  
20 25 30

Ala Glu Ala Ile Ala Pro Gln Val Gly Asn Asp Ala Val Phe Leu Ile  
35 40 45

Leu Tyr Lys Glu Leu Tyr Tyr Arg His Ile Tyr Ala Lys Val Ser Gly  
50 55 60

Gly Pro Ser Leu Glu Gln Arg Phe Glu Ser Tyr Tyr Asn Tyr Cys Asn  
65 70 75 80

Leu Phe Asn Tyr Ile Leu Asn Ala Asp Gly Pro Ala Pro Leu Glu Leu  
85 90 95

Pro Asn Gln Trp Leu Trp Asp Ile Ile Asp Glu Phe Ile Tyr Gln Phe  
100 105 110

Gln Ser Phe Ser Gln Tyr Arg Cys Lys Thr Ala Lys Lys Ser Glu Glu  
115 120 125

Glu Ile Asp Phe Leu Arg Ser Asn Pro Lys Ile Trp Asn Val His Ser  
130 135 140

Val Leu Asn Val Leu His Ser Leu Val Asp Lys Ser Asn Ile Asn Arg  
145 150 155 160

Gln Leu Glu Val Tyr Thr Ser Gly Gly Asp Pro Glu Ser Val Ala Gly  
165 170 175

Glu Tyr Gly Arg His Ser Leu Tyr Lys Met Leu Gly Tyr Phe Ser Leu  
180 185 190

Val Gly Leu Leu Arg Leu His Ser Leu Leu Gly Asp Tyr Tyr Gln Ala  
195 200 205

Ile Lys Val Leu Glu Asn Ile Glu Leu Asn Lys Lys Ser Met Tyr Ser  
 210 215 220  
 Arg Val Pro Glu Cys Gln Val Thr Thr Tyr Tyr Val Gly Phe Ala  
 225 230 235 240  
 Tyr Leu Met Met Arg Arg Tyr Gln Asp Ala Ile Arg Val Phe Ala Asn  
 245 250 255  
 Ile Leu Leu Tyr Ile Gln Arg Thr Lys Ser Met Phe Gln Arg Thr Thr  
 260 265 270  
 Tyr Lys Tyr Glu Met Ile Asn Lys Gln Asn Glu Gln Met His Ala Leu  
 275 280 285  
 Leu Ala Ile Ala Leu Thr Met Tyr Pro Met Arg Ile Asp Glu Ser Ile  
 290 295 300  
 His Leu Gln Leu Arg Glu Lys Tyr Gly Asp Lys Met Leu Arg Met Gln  
 305 310 315 320  
 Lys Gly Asp Pro Gln Val Tyr Glu Glu Leu Phe Ser Tyr Ser Cys Pro  
 325 330 335  
 Lys Phe Leu Ser Pro Val Val Pro Asn Tyr Asp Asn Val His Pro Asn  
 340 345 350  
 Tyr His Lys Glu Pro Phe Leu Gln Gln Leu Lys Val Phe Ser Asp Glu  
 355 360 365  
 Val Gln Gln Gln Ala Gln Leu Ser Thr Ile Arg Ser Phe Leu Lys Leu  
 370 375 380  
 Tyr Thr Thr Met Pro Val Ala Lys Leu Ala Gly Phe Leu Asp Leu Thr  
 385 390 395 400  
 Glu Gln Glu Phe Arg Ile Gln Leu Leu Val Phe Lys His Lys Met Lys  
 405 410 415  
 Asn Leu Val Trp Thr Ser Gly Ile Ser Ala Leu Asp Gly Glu Phe Gln  
 420 425 430  
 Ser Ala Ser Glu Val Asp Phe Tyr Ile Asp Lys Asp Met Ile His Ile  
 435 440 445  
 Ala Asp Thr Lys Val Ala Arg Arg Tyr Gly  
 450 455

&lt;210&gt; 119

&lt;211&gt; 356

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 119

Ile Lys Phe Leu Glu Val Ile Lys Pro Phe Cys Val Ile Leu Pro Glu  
 1 5 10 15

Ile Gln Lys Pro Glu Arg Lys Ile Gln Phe Lys Glu Lys Val Leu Trp  
 20 25 30  
 Thr Ala Ile Thr Leu Phe Ile Phe Leu Val Cys Cys Gln Ile Pro Leu  
 35 40 45  
 Phe Gly Ile Met Ser Ser Asp Ser Ala Asp Pro Phe Tyr Trp Met Arg  
 50 55 60  
 Val Ile Leu Ala Ser Asn Arg Gly Thr Leu Met Glu Leu Gly Ile Ser  
 65 70 75 80  
 Pro Ile Val Thr Ser Gly Leu Ile Met Gln Leu Leu Ala Gly Ala Lys  
 85 90 95  
 Ile Ile Glu Val Gly Asp Thr Pro Lys Asp Arg Ala Leu Phe Asn Gly  
 100 105 110  
 Ala Gln Lys Leu Phe Gly Met Ile Ile Thr Ile Gly Gln Ser Ile Val  
 115 120 125  
 Tyr Val Met Thr Gly Met Tyr Gly Asp Pro Ser Glu Met Gly Ala Gly  
 130 135 140  
 Ile Cys Leu Leu Ile Thr Ile Gln Leu Phe Val Ala Gly Leu Ile Val  
 145 150 155 160  
 Leu Leu Leu Asp Glu Leu Leu Gln Lys Gly Tyr Gly Leu Gly Ser Gly  
 165 170 175  
 Ile Ser Leu Phe Ile Ala Thr Asn Ile Cys Glu Thr Ile Val Trp Lys  
 180 185 190  
 Ala Phe Ser Pro Thr Thr Val Asn Thr Gly Arg Gly Met Glu Phe Glu  
 195 200 205  
 Gly Ala Ile Ile Ala Leu Phe His Leu Leu Ala Thr Arg Thr Asp Lys  
 210 215 220  
 Val Arg Ala Leu Arg Glu Ala Phe Tyr Arg Gln Asn Leu Pro Asn Leu  
 225 230 235 240  
 Met Asn Leu Ile Ala Thr Ile Phe Val Phe Ala Val Val Ile Tyr Phe  
 245 250 255  
 Gln Gly Phe Arg Val Asp Leu Pro Ile Lys Ser Ala Arg Tyr Arg Gly  
 260 265 270  
 Gln Tyr Asn Thr Tyr Pro Ile Lys Leu Phe Tyr Thr Ser Asn Ile Pro  
 275 280 285  
 Ile Ile Leu Gln Ser Ala Leu Val Ser Asn Leu Tyr Val Ile Ser Gln  
 290 295 300  
 Met Leu Ser Ala Arg Phe Ser Gly Asn Leu Leu Val Ser Leu Leu Gly  
 305 310 315 320

Thr Trp Ser Asp Thr Ser Ser Gly Gly Pro Ala Arg Ala Tyr Pro Val  
 325 330 335

Gly Gly Leu Cys Tyr Tyr Leu Ser Pro Pro Glu Ser Phe Gly Ser Val  
 340 345 350

Leu Glu Asp Pro  
 355

<210> 120

<211> 356

<212> PRT

<213> Homo sapiens

<400> 120

Ile Lys Phe Leu Glu Val Ile Lys Pro Phe Cys Val Ile Leu Pro Glu  
 1 5 10 15

Ile Gln Lys Pro Glu Arg Lys Ile Gln Phe Lys Glu Lys Val Leu Trp  
 20 25 30

Thr Ala Ile Thr Leu Phe Ile Phe Leu Val Cys Cys Gln Ile Pro Leu  
 35 40 45

Phe Gly Ile Met Ser Ser Asp Ser Ala Asp Pro Phe Tyr Trp Met Arg  
 50 55 60

Val Ile Leu Ala Ser Asn Arg Gly Thr Leu Met Glu Leu Gly Ile Ser  
 65 70 75 80

Pro Ile Val Thr Ser Gly Leu Ile Met Gln Leu Leu Ala Gly Ala Lys  
 85 90 95

Ile Ile Glu Val Gly Asp Thr Pro Lys Asp Arg Ala Leu Phe Asn Gly  
 100 105 110

Ala Gln Lys Leu Phe Gly Met Ile Ile Thr Ile Gly Gln Ser Ile Val  
 115 120 125

Tyr Val Met Thr Gly Met Tyr Gly Asp Pro Ser Glu Met Gly Ala Gly  
 130 135 140

Ile Cys Leu Leu Ile Thr Ile Gln Leu Phe Val Ala Gly Leu Ile Val  
 145 150 155 160

Leu Leu Leu Asp Glu Leu Leu Gln Lys Gly Tyr Gly Leu Gly Ser Gly  
 165 170 175

Ile Ser Leu Phe Ile Ala Thr Asn Ile Cys Glu Thr Ile Val Trp Lys  
 180 185 190

Ala Phe Ser Pro Thr Thr Val Asn Thr Gly Arg Gly Met Glu Phe Glu  
 195 200 205

Gly Ala Ile Ile Ala Leu Phe His Leu Leu Ala Thr Arg Thr Asp Lys  
 210 215 220

Val Arg Ala Leu Arg Glu Ala Phe Tyr Arg Gln Asn Leu Pro Asn Leu  
225 230 235 240

Met Asn Leu Ile Ala Thr Ile Phe Val Phe Ala Val Val Ile Tyr Phe  
245 250 255

Gln Gly Phe Arg Val Asp Leu Pro Ile Lys Ser Ala Arg Tyr Arg Gly  
260 265 270

Gln Tyr Asn Thr Tyr Pro Ile Lys Leu Phe Tyr Thr Ser Asn Ile Pro  
275 280 285

Ile Ile Leu Gln Ser Ala Leu Val Ser Asn Leu Tyr Val Ile Ser Gln  
290 295 300

Met Leu Ser Ala Arg Phe Ser Gly Asn Leu Leu Val Ser Leu Leu Gly  
305 310 315 320

Thr Trp Ser Asp Thr Ser Ser Gly Gly Pro Ala Arg Ala Tyr Pro Val  
325 330 335

Gly Gly Leu Cys Tyr Tyr Leu Ser Pro Pro Trp Ser Met Asn Ser Thr  
340 345 350

Gly Thr Ser Pro  
355

<210> 121

<211> 68

<212> PRT

<213> Homo sapiens

<400> 121

Met Val His Glu Leu Asn Arg Tyr Ile Pro Thr Ala Ala Ala Phe Gly  
1 5 10 15

Gly Leu Cys Ile Gly Ala Leu Ser Val Leu Ala Asp Phe Leu Gly Ala  
20 25 30

Ile Gly Ser Gly Thr Gly Ile Leu Leu Ala Val Thr Ile Ile Tyr Gln  
35 40 45

Tyr Phe Glu Ile Phe Val Lys Glu Gln Ser Glu Val Gly Ser Met Gly  
50 55 60

Ala Leu Leu Phe  
65

<210> 122

<211> 68

<212> PRT

<213> Homo sapiens

<400> 122

Met Val His Glu Leu Asn Arg Tyr Ile Pro Thr Ala Ala Ala Phe Gly  
1 5 10 15

Gly Leu Cys Ile Gly Ala Leu Ser Val Leu Ala Asp Phe Leu Gly Ala  
                   20                  25                  30  
 Ile Gly Ser Gly Thr Gly Ile Leu Ala Val Thr Ile Ile Tyr Gln  
                   35                  40                  45  
 Tyr Phe Glu Ile Phe Val Lys Glu Gln Ser Glu Val Gly Ser Met Gly  
                   50                  55                  60  
 Ala Leu Leu Phe  
                   65  
  
 <210> 123  
 <211> 224  
 <212> PRT  
 <213> Homo sapiens  
  
 <400> 123  
 Leu Pro Asn Leu Val Asn Asn Phe Glu Lys Tyr Leu Ser Phe Ser Pro  
                   1                  5                  10                  15  
 His Ile Arg Glu Pro Thr Ile His Phe Val Leu Tyr Val Pro Ser Pro  
                   20                  25                  30  
 Gln Ile Gln Pro Leu Trp Leu Glu Asn Glu Asp Ser Asn Ile Ile Pro  
                   35                  40                  45  
 Thr Asn Ser Met Leu Leu Pro Gln Trp Gly Ser Ile Thr Thr Ile Asn  
                   50                  55                  60  
 Phe Asn Val Thr Glu Lys Lys Leu Leu His Asp Val Asp Leu Lys Asp  
                   65                  70                  75                  80  
 Tyr Phe Arg Val Ile Ser Arg Asp Leu Leu Leu Leu Gly Ile Asn  
                   85                  90                  95  
 Asp Val Pro Val Ser Ser Leu Ser Ala Thr Leu Ala Asp Arg Leu Leu  
                   100                  105                  110  
 Arg Gln Arg Ile Ala Glu Ser Cys Ile Glu Ala Ser Asp Thr Leu Gln  
                   115                  120                  125  
 Asn Leu Ala Lys Leu Val His Ser Met Gln Ser Met Ala Val Pro Lys  
                   130                  135                  140  
 Glu Ile Gln Met Tyr Val Lys Asp Thr Leu Leu Ser Leu Asp Met Ala  
                   145                  150                  155                  160  
 Tyr Lys Ala Leu Ser Gln Asn Asn Leu Asn Glu Ala Leu Ser Tyr Ser  
                   165                  170                  175  
 Asn Asn Ala Phe Ser Lys Ser Gln Glu Ala Leu Phe His Pro Ser Met  
                   180                  185                  190  
 Val Thr Thr Ile Tyr Phe Pro Asp Glu Ser Lys Tyr Gly Ile Tyr Ala  
                   195                  200                  205

Pro Leu Phe Ala Pro Ile Leu Ile Pro Leu Leu Ile Ser Phe Ile Lys  
 210 215 220

<210> 124  
 <211> 240  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (148)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 124  
 Leu Pro His Val Ile Asn Pro Val Glu Ser Arg Leu Gly Ser Ser Ala  
 1 5 10 15  
 Ala Ser Leu Tyr Pro Val Leu Asn Phe Leu Leu Tyr Val Pro Glu Leu  
 20 25 30  
 Ala His Ser Pro Leu Tyr Ile Gln Asp Lys Asp Gly Ala Pro Val Ala  
 35 40 45  
 Thr Asn Ala Phe His Ser Pro Arg Trp Gly Gly Ile Met Val Tyr Asn  
 50 55 60  
 Val Asp Ser Lys Thr Tyr Asn Ala Ser Val Leu Pro Val Arg Val Glu  
 65 70 75 80  
 Val Asp Met Val Arg Val Met Glu Val Phe Leu Ala Gln Leu Arg Leu  
 85 90 95  
 Leu Phe Gly Ile Ala Gln Pro Gln Leu Pro Pro Lys Cys Leu Leu Ser  
 100 105 110  
 Gly Pro Thr Ser Glu Gly Leu Met Thr Trp Glu Leu Asp Arg Leu Leu  
 115 120 125  
 Trp Ala Arg Ser Val Glu Asn Leu Ala Thr Ala Thr Thr Thr Leu Thr  
 130 135 140  
 Ser Leu Ala Xaa Phe Leu Gly Lys Ile Ser Asn Ile Val Ile Lys Asp  
 145 150 155 160  
 Asp Val Ala Ser Glu Val Tyr Lys Ala Val Ala Ala Val Gln Lys Ser  
 165 170 175  
 Ala Glu Glu Leu Ala Ser Gly His Leu Ala Ser Ala Phe Val Ala Ser  
 180 185 190  
 Gln Glu Ala Val Thr Ser Ser Glu Leu Ala Phe Phe Asp Pro Ser Leu  
 195 200 205



81

Leu His Leu Leu Tyr Phe Pro Asp Asp Gln Lys Phe Ala Ile Tyr Ile  
 210 215 220

Pro Leu Phe Leu Pro Met Ala Val Pro Ile Leu Leu Ser Leu Val Lys  
 225 230 235 240

&lt;210&gt; 125

&lt;211&gt; 101

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 125

Tyr Phe Cys Ser Ile Ala Leu Ser Ile Tyr Asn Arg Trp Met Phe Asp  
 1 5 10 15

Pro Lys Asp Gly Leu Gly Ile Gly Tyr Pro Val Leu Val Thr Thr Phe  
 20 25 30

His Gln Ala Thr Leu Trp Leu Leu Ser Gly Ile Tyr Ile Lys Leu Arg  
 35 40 45

His Lys Pro Val Lys Asn Val Leu Arg Lys Asn Asn Gly Phe Asn Trp  
 50 55 60

Ser Phe Phe Leu Lys Phe Leu Leu Pro Thr Ala Val Ala Ser Ala Gly  
 65 70 75 80

Asp Ile Gly Leu Ser Asn Val Ser Phe Gln Tyr Val Pro Leu Thr Ile  
 85 90 95

Tyr Thr Ile Ile Lys  
 100

&lt;210&gt; 126

&lt;211&gt; 94

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 126

Tyr Tyr Cys Phe Ser Ile Gly Ile Thr Phe Tyr Asn Lys Trp Leu Thr  
 1 5 10 15

Lys Ser Phe His Phe Pro Leu Phe Met Thr Met Leu His Leu Ala Val  
 20 25 30

Ile Phe Leu Phe Ser Ala Leu Ser Arg Ala Leu Val Gln Cys Ser Ser  
 35 40 45

His Arg Ala Arg Val Val Leu Ser Trp Ala Asp Tyr Leu Arg Arg Val  
 50 55 60

Ala Pro Thr Ala Leu Ala Thr Ala Leu Asp Val Gly Leu Ser Asn Trp  
 65 70 75 80

Ser Phe Leu Tyr Val Thr Val Ser Leu Tyr Thr Met Thr Lys  
 85 90

<210> 127

<211> 250

<212> PRT

<213> Homo sapiens

<400> 127

Ser Lys Glu Gln Ser Ile Leu Gln Lys Ser Ala Ile Trp Pro Ser Tyr  
 1 5 10 15

Val Arg Glu Arg Ile Ser Thr Thr Tyr Ala Tyr Leu Ala Gly Ser Leu  
 20 25 30

Ala Leu Thr Ala Val Ser Gly Val Ala Ala Ser Arg Ser Ala Ala Ile  
 35 40 45

Met Arg Leu Thr Ala Gly Gly Gly Met Met Ser Leu Phe Gly Thr Met  
 50 55 60

Ala Ala Met Ile Ala Ser Gly Met Leu Ala Arg Ser Ile Asp Tyr Glu  
 65 70 75 80

Ser Thr Val Ala Lys His Leu Ala Trp Ala Leu His Cys Gly Val Leu  
 85 90 95

Gly Ala Val Phe Ala Pro Leu Cys Phe Met Ala Gly Pro Val Leu Thr  
 100 105 110

Arg Ala Ala Trp Tyr Thr Ala Gly Ile Val Gly Gly Leu Ser Ala Thr  
 115 120 125

Ala Ile Thr Ala Pro Ser Glu Lys Phe Leu Met Met Ser Gly Pro Leu  
 130 135 140

Ala Met Gly Phe Gly Val Val Phe Val Ala Asn Ile Gly Ala Phe Phe  
 145 150 155 160

Leu Pro Pro Gly Ser Ala Leu Gly Ala Ser Leu Ala Ser Ile Val Val  
 165 170 175

Tyr Gly Gly Leu Ile Leu Phe Ser Ala Phe Leu Leu Tyr Asp Thr Gln  
 180 185 190

Arg Leu Val Lys Lys Ala Glu Asn His Pro His Ser Ser Gln Leu Tyr  
 195 200 205

Gly Ser Asp Met Gln Ile Arg Ser Phe Asp Pro Ile Asn Ala Gln Met  
 210 215 220

Ser Ile Tyr Met Asp Val Leu Asn Ile Phe Met Arg Leu Val Met Ile  
 225 230 235 240

Met Gly Gly Met Asn Gly Asn Arg Arg Lys  
 245 250

&lt;210&gt; 128

&lt;211&gt; 242

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 128

Ser Asn Glu Ile Gly Ala Ile Glu Lys Ala Val Ile Trp Pro Gln Tyr  
 1 5 10 15

Val Lys Asp Arg Ile His Ser Thr Tyr Met Tyr Leu Ala Gly Ser Ile  
 20 25 30

Gly Leu Thr Ala Leu Ser Ala Ile Ala Ile Ser Arg Thr Pro Val Leu  
 35 40 45

Met Asn Phe Met Met Arg Gly Ser Trp Val Thr Ile Gly Val Thr Phe  
 50 55 60

Ala Ala Met Val Gly Ala Gly Met Leu Val Arg Ser Ile Pro Tyr Asp  
 65 70 75 80

Gln Ser Pro Gly Pro Lys His Leu Ala Trp Leu Leu His Ser Gly Val  
 85 90 95

Met Gly Ala Val Val Ala Pro Leu Thr Ile Leu Gly Gly Pro Leu Leu  
 100 105 110

Ile Arg Ala Ala Trp Tyr Thr Ala Gly Ile Val Gly Gly Leu Ser Thr  
 115 120 125

Val Ala Met Cys Ala Pro Ser Glu Lys Phe Leu Asn Met Gly Ala Pro  
 130 135 140

Leu Gly Val Gly Leu Gly Leu Val Phe Val Ser Ser Leu Gly Ser Met  
 145 150 155 160

Phe Leu Pro Pro Thr Thr Val Ala Gly Ala Thr Leu Tyr Ser Val Ala  
 165 170 175

Met Tyr Gly Gly Leu Val Leu Phe Ser Met Phe Leu Leu Tyr Asp Thr  
 180 185 190

Gln Lys Val Ile Lys Arg Ala Glu Val Ser Pro Met Tyr Gly Val Gln  
 195 200 205

Lys Tyr Asp Pro Ile Asn Ser Met Leu Ser Ile Tyr Met Asp Thr Leu  
 210 215 220

Asn Ile Phe Met Arg Val Ala Thr Met Leu Ala Thr Gly Gly Asn Arg  
 225 230 235 240

Lys Lys

&lt;210&gt; 129

&lt;211&gt; 179

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 129

Met Gly Ile Leu Phe Thr Arg Ile Trp Arg Leu Phe Asn His Gln Glu  
 1 5 10 15

His Lys Val Ile Ile Val Gly Leu Asp Asn Ala Gly Lys Thr Thr Ile  
 20 25 30

Leu Tyr Gln Phe Ser Met Asn Glu Val Val His Thr Ser Pro Thr Ile  
 35 40 45

Gly Ser Asn Val Glu Glu Ile Val Val Asn Asn Thr Arg Phe Leu Met  
 50 55 60

Trp Asp Ile Gly Gly Gln Glu Ser Leu Arg Ser Ser Trp Asn Thr Tyr  
 65 70 75 80

Tyr Thr Asn Thr Glu Phe Val Ile Val Val Val Asp Ser Thr Asp Arg  
 85 90 95

Glu Arg Ile Ser Val Thr Arg Glu Glu Leu Tyr Lys Met Leu Ala His  
 100 105 110

Glu Asp Leu Arg Lys Ala Gly Leu Leu Ile Phe Ala Asn Lys Gln Asp  
 115 120 125

Val Lys Glu Cys Met Thr Val Ala Glu Ile Ser Gln Phe Leu Lys Leu  
 130 135 140

Thr Ser Ile Lys Asp His Gln Trp His Ile Gln Ala Cys Cys Ala Leu  
 145 150 155 160

Thr Gly Glu Gly Leu Cys Gln Gly Leu Glu Trp Met Met Ser Arg Leu  
 165 170 175

Lys Ile Arg

&lt;210&gt; 130

&lt;211&gt; 179

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 130

Met Gly Leu Ile Phe Ala Lys Leu Trp Ser Leu Phe Cys Asn Gln Glu  
 1 5 10 15

His Lys Val Ile Ile Val Gly Leu Asp Asn Ala Gly Lys Thr Thr Ile  
 20 25 30

Leu Tyr Gln Phe Leu Met Asn Glu Val Val His Thr Ser Pro Thr Ile  
 35 40 45

Gly Ser Asn Val Glu Glu Ile Val Val Lys Asn Thr His Phe Leu Met

85

50                      55                      60  
 Trp Asp Ile Gly Gly Gln Glu Ser Leu Arg Ser Ser Trp Asn Thr Tyr  
 65                      70                      75                      80  
 Tyr Ser Asn Thr Glu Phe Ile Ile Leu Val Val Asp Ser Ile Asp Arg  
                     85                      90                      95  
 Glu Arg Leu Ala Ile Thr Lys Glu Glu Leu Tyr Arg Met Leu Ala His  
                     100                      105                      110  
 Glu Asp Leu Arg Lys Ala Ala Val Leu Ile Phe Ala Asn Lys Gln Asp  
                     115                      120                      125  
 Met Lys Gly Cys Met Thr Ala Ala Glu Ile Ser Lys Tyr Leu Thr Leu  
                     130                      135                      140  
 Ser Ser Ile Lys Asp His Pro Trp His Ile Gln Ser Cys Cys Ala Leu  
                     145                      150                      155                      160  
 Thr Gly Glu Gly Leu Cys Gln Gly Leu Glu Trp Met Thr Ser Arg Ile  
                     165                      170                      175  
 Gly Val Arg

&lt;210&gt; 131

&lt;211&gt; 379

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 131

Arg Arg Phe Phe Asn Trp Phe Tyr Trp Ser Ile Asn Leu Gly Ala Ile  
 1                      5                      10                      15  
 Leu Ser Leu Gly Gly Ile Ala Tyr Ile Gln Gln Asn Val Ser Phe Leu  
                     20                      25                      30  
 Thr Gly Tyr Leu Ile Pro Thr Val Cys Val Ala Ile Ala Phe Leu Val  
                     35                      40                      45  
 Phe Leu Cys Gly Gln Ser Val Phe Ile Thr Lys Pro Pro Asp Gly Ser  
                     50                      55                      60  
 Ala Phe Thr Asp Met Phe Arg Ile Leu Thr Tyr Ser Cys Cys Ser Gln  
                     65                      70                      75                      80  
 Arg Gly Gly Gln Arg Arg Ser Gly Glu Gly Leu Gly Val Phe Gln Gln  
                     85                      90                      95  
 Ser Ser Lys His Ser Leu Phe Asp Ser Cys Lys Met Ser Arg Gly Gly  
                     100                      105                      110  
 Pro Phe Thr Glu Asp Lys Val Glu Asp Val Lys Ala Leu Val Lys Ile  
                     115                      120                      125  
 Val Pro Val Phe Leu Ala Leu Ile Pro Tyr Trp Thr Val Tyr Phe Gln

|                                                                 |     |             |
|-----------------------------------------------------------------|-----|-------------|
| 130                                                             | 135 | 140         |
| Met Gln Thr Thr Tyr Val Leu Gln Ser Leu His Leu Lys Ile Pro Glu |     |             |
| 145                                                             | 150 | 155 160     |
| Ile Ser Ser Ile Thr Thr Thr His His Thr Leu Pro Ala Ala Trp Leu |     |             |
|                                                                 | 165 | 170 175     |
| Thr Met Phe Asp Ala Val Leu Ile Leu Leu Leu Ile Pro Leu Lys Asp |     |             |
|                                                                 | 180 | 185 190     |
| Lys Leu Val Asp Pro Val Leu Arg Arg His Gly Leu Leu Pro Ser Ser |     |             |
|                                                                 | 195 | 200 205     |
| Leu Lys Arg Ile Ala Val Gly Met Phe Phe Val Thr Cys Ser Ala Phe |     |             |
|                                                                 | 210 | 215 220     |
| Ala Ala Gly Ile Leu Glu Ser Lys Arg Leu Asp Leu Val Lys Glu Lys |     |             |
|                                                                 | 225 | 230 235 240 |
| Thr Ile Asn Gln Thr Ile Gly Gly Val Val Tyr His Ala Ala Asp Leu |     |             |
|                                                                 | 245 | 250 255     |
| Pro Ile Trp Trp Gln Ile Pro Gln Tyr Val Leu Ile Gly Ile Ser Glu |     |             |
|                                                                 | 260 | 265 270     |
| Ile Phe Ala Ser Ile Ala Gly Leu Glu Phe Ala Tyr Ser Ala Ala Pro |     |             |
|                                                                 | 275 | 280 285     |
| Lys Ser Met Gln Ser Ala Ile Met Gly Leu Phe Phe Phe Phe Ser Gly |     |             |
|                                                                 | 290 | 295 300     |
| Ile Gly Ser Phe Val Gly Ser Gly Leu Leu Ala Leu Val Ser Leu Lys |     |             |
|                                                                 | 305 | 310 315 320 |
| Ala Ile Gly Trp Met Ser Ser His Thr Asp Phe Gly Asn Ile Asn Ser |     |             |
|                                                                 | 325 | 330 335     |
| Cys His Leu His Tyr Tyr Phe Phe Leu Leu Ala Ala Ile Gln Gly Ala |     |             |
|                                                                 | 340 | 345 350     |
| Thr Leu Leu Leu Phe Leu Ile Val Ser Val Lys Tyr Asp Arg Gln Arg |     |             |
|                                                                 | 355 | 360 365     |
| Ala Arg Thr Asp Gly Gly Thr Ala Ser Thr Arg                     |     |             |
|                                                                 | 370 | 375         |

&lt;210&gt; 132

&lt;211&gt; 385

&lt;212&gt; FRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (48)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 132

```

Arg Arg Phe Phe Asn Trp Phe Tyr Trp Ser Ile Asn Leu Gly Ala Ile
 1 5 10 15

Leu Ser Leu Gly Gly Ile Ala Tyr Ile Gln Gln Asn Val Ser Phe Val
 20 25 30

Thr Gly Tyr Ala Ile Pro Thr Val Cys Val Gly Leu Ala Phe Val Xaa
 35 40 45

Phe Leu Cys Gly Gln Ser Val Phe Ile Thr Lys Pro Pro Asp Gly Ser
 50 55 60

Ala Phe Thr Asp Met Phe Lys Ile Leu Thr Tyr Ser Cys Cys Ser Gln
 65 70 75 80

Lys Arg Ser Gly Glu Arg Gln Ser Asn Gly Glu Gly Ile Gly Val Phe
 85 90 95

Gln Gln Ser Ser Lys Gln Ser Leu Phe Asp Ser Cys Lys Met Ser His
100 105 110

Gly Gly Pro Phe Thr Glu Glu Lys Val Glu Asp Val Lys Ala Leu Val
115 120 125

Lys Ile Val Pro Val Phe Leu Ala Leu Ile Pro Tyr Trp Thr Val Tyr
130 135 140

Phe Gln Met Gln Thr Thr Tyr Val Leu Gln Ser Leu His Leu Arg Ile
145 150 155 160

Pro Glu Ile Ser Asn Ile Thr Thr Thr Pro His Thr Leu Pro Ala Ala
165 170 175

Trp Leu Thr Met Phe Asp Ala Val Leu Ile Leu Leu Leu Ile Pro Leu
180 185 190

Lys Asp Lys Leu Val Asp Pro Ile Leu Arg Arg His Gly Leu Leu Pro
195 200 205

Ser Ser Leu Lys Arg Ile Ala Val Gly Met Phe Phe Val Met Cys Ser
210 215 220

Ala Phe Ala Ala Gly Ile Leu Glu Ser Lys Arg Leu Asn Leu Val Lys
225 230 235 240

Glu Lys Thr Ile Asn Gln Thr Ile Gly Asn Val Val Tyr His Ala Ala
245 250 255

Asp Leu Ser Leu Trp Trp Gln Val Pro Gln Tyr Leu Leu Ile Gly Ile
260 265 270

Ser Glu Ile Phe Ala Ser Ile Ala Gly Leu Glu Phe Ala Tyr Ser Ala
275 280 285

Ala Pro Lys Ser Met Gln Ser Ala Ile Met Gly Leu Phe Phe Phe Phe
290 295 300

```

Ser Gly Val Gly Ser Phe Val Gly Ser Gly Leu Leu Ala Leu Val Ser  
305 310 315 320

Ile Lys Ala Ile Gly Trp Met Ser Ser His Thr Asp Phe Gly Asn Ile  
325 330 335

Asn Gly Cys Tyr Leu Asn Tyr Tyr Phe Phe Leu Leu Ala Ala Ile Gln  
340 345 350

Gly Ala Thr Leu Leu Leu Phe Leu Ile Ile Ser Val Lys Tyr Asp His  
355 360 365

His Arg Asp His Gln Arg Ser Arg Ala Asn Gly Val Pro Thr Ser Arg  
370 375 380

Arg  
385

<210> 133

<211> 92

<212> PRT

<213> Homo sapiens

<400> 133

Leu Asn Thr Gln Leu Phe Thr Gln Thr Ser Pro Val Ala Asp Ala Pro  
1 5 10 15

Thr Gly Val Gln Trp His Asp Phe Gly Ser Leu Gln Pro Leu Pro Pro  
20 25 30

Gly Phe Lys Arg Phe Ser Cys Leu Ser Leu Pro Arg Ser Trp Asp Tyr  
35 40 45

Arg His Pro Pro Pro Arg Pro Ala Asn Phe Glu Phe Leu Val Glu Thr  
50 55 60

Gly Phe Leu His Val Gly Gln Ala Gly Leu Glu Leu Leu Thr Ser Gly  
65 70 75 80

Asp Leu Pro Ala Ser Ala Ser Gln Ser Ala Arg Ile  
85 90

<210> 134

<211> 94

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (15)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<220>

<221> SITE

<222> (27)

<223> Xaa equals any of the twenty naturally occurring L-amino acids



```
<220>
<221> SITE
<222> (35)
<223> Xaa equals any of the twenty naturally occurring L-amino acids
```

<400> 134  
Leu Ser Leu Ser Leu Phe Glu Met Glu Ser Cys Leu Val Ala Xaa Ala  
1 5 10 15

Gly Val Gln Trp His Asp Leu Gly Ser Leu Xaa Pro Pro Pro Leu Gly  
20 25 30

Phe Lys Xaa Phe Ser Cys Leu Ser Leu Pro Asn Arg Trp Asp Tyr Lys  
35 40 45

Val Cys Thr Thr Lys Pro Gly Gln Phe Phe Phe Phe Val Phe Leu Val  
50 55 60

Glu Met Gly Phe His His Ile Gly Gln Ala Gly Leu Glu Leu Leu Thr  
65 70 75 80

Ser Asn Asp Arg Ser Ala Ser Ala Ser Gln Ser Ala Gly Ile  
85 90

```
<210> 135
<211> 49
<212> PRT
<213> Homo sapiens
```

<400> 135  
Gln Ala Gly His Gly Ala Leu Thr Cys Asn Pro Ser Thr Leu Gly Arg  
1 5 10 15

Pro Arg Gln Val Asp His Leu Arg Ser Gly Val Arg Asp Gln Pro Gly  
20 25 30

Gln His Gly Lys Thr Pro Ser Thr Arg Asn Thr Lys Ile Ser Gly Arg  
35 40 45

Gly

```
<210> 136
<211> 50
<212> PRT
<213> Homo sapiens
```

```
<220>
<221> SITE
<222> (10)
<223> Xaa equals any of the twenty naturally occurring L-amino acids
```

<400> 136  
Lys Ala Gly Ser Gly Gly Ser Cys Leu Xaa Ser Arg His Leu Gly Arg  
1 5 10 15

Pro Arg Trp Val Asp His Leu Arg Ser Gly Val Arg Asp Gln Pro Asp  
                   20                  25                  30

Gln Tyr Gly Glu Thr Pro Ile Ser Thr Lys Asn Thr Lys Ile Ser Ser  
                   35                  40                  45

Gly Gly  
           50

<210> 137

<211> 325

<212> PRT

<213> Homo sapiens

<400> 137

Gln Asn Gly Asn Glu Gln Val Ile, Lys Asp Val Arg Lys Ile Thr Arg  
           1                  5                  10                  15

Ser Gly Asp Asp Trp Ile Pro Asp Ser Pro Gln Asp Leu Ala Ser Lys  
                   20                  25                  30

Ile Phe His Ser Cys Phe Met Gly Thr Glu Asn Ser Ser Lys Glu Thr  
                   35                  40                  45

Arg Asn Arg Ala Lys Asp Leu Ser Asn Ala Ile Gly Ser Tyr His Val  
                   50                  55                  60

Asp Leu Lys Met Asp Ser Leu Val Ser Ser Val Val Ser Leu Phe Glu  
                   65                  70                  75                  80

Val Ala Thr Gly Lys Lys Pro Ile Tyr Lys Ile Phe Gly Gly Ser Gln  
                   85                  90                  95

Ile Glu Asn Leu Ala Leu Gln Asn Ile Gln Ala Arg Leu Arg Met Val  
                   100                  105                  110

Leu Ser Tyr Leu Phe Ala Gln Leu Leu Pro Trp Val Arg Gly Ile Pro  
                   115                  120                  125

Asn Ser Gly Gly Leu Leu Val Leu Gly Ser Ala Asn Val Asp Glu Cys  
                   130                  135                  140

Leu Arg Gly Tyr Leu Thr Lys Tyr Asp Cys Ser Ser Ala Asp Ile Asn  
                   145                  150                  155                  160

Pro Ile Gly Gly Ile Ser Lys Thr Asp Leu Lys Arg Phe Ile Ala Tyr  
                   165                  170                  175

Ala Ser Lys Gln Tyr Asn Met Pro Ile Leu Asn Asp Phe Leu Asn Ala  
                   180                  185                  190

Thr Pro Thr Ala Glu Leu Glu Pro Met Thr Lys Asp Tyr Val Gln Ser  
                   195                  200                  205

Asp Glu Ile Asp Met Gly Met Thr Tyr Glu Glu Leu Gly Val Phe Gly  
                   210                  215                  220

Tyr Leu Arg Lys Val Glu Lys Cys Gly Pro Tyr Ser Met Phe Leu Lys  
 225 230 235 240  
 Leu Leu His Gln Trp Ser Pro Lys Leu Thr Pro Arg Gln Ile Ser Glu  
 245 250 255  
 Lys Val Lys Arg Phe Phe Phe Phe Tyr Ala Ile Asn Arg His Lys Gln  
 260 265 270  
 Thr Val Leu Thr Pro Ser Tyr His Ala Glu Gln Tyr Ser Pro Glu Asp  
 275 280 285  
 Asn Arg Phe Asp Leu Arg Pro Phe Leu Ile Asn Pro Arg Phe Pro Trp  
 290 295 300  
 Ala Ser Arg Lys Ile Asp Glu Val Val Glu Gln Cys Glu Ala His Lys  
 305 310 315 320  
 Gly Ser Thr Leu Asp  
 325

&lt;210&gt; 138

&lt;211&gt; 323

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (21)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 138

Arg Ser Gly Asn Glu Glu Val Leu Ala Asp Val Arg Thr Ile Val Asn  
 1 5 10 15

Gln Ile Ser Tyr Xaa Pro Gln Asp Pro Arg Asp Leu Cys Gly Arg Ile  
 20 25 30

Leu Thr Thr Cys Tyr Met Ala Ser Lys Asn Ser Ser Gln Glu Thr Cys  
 35 40 45

Thr Arg Ala Arg Glu Leu Ala Gln Gln Ile Gly Ser His His Ile Ser  
 50 55 60

Leu Asn Ile Asp Pro Ala Val Lys Ala Val Met Gly Ile Phe Ser Leu  
 65 70 75 80

Val Thr Gly Lys Ser Pro Leu Phe Ala Ala His Gly Gly Ser Ser Arg  
 85 90 95

Glu Asn Leu Ala Leu Gln Asn Val Gln Ala Arg Ile Arg Met Val Leu  
 100 105 110

Ala Tyr Leu Phe Ala Gln Leu Ser Leu Trp Ser Arg Gly Val His Gly  
 115 120 125

Gly Leu Leu Val Leu Gly Ser Ala Asn Val Asp Glu Ser Leu Leu Gly  
 130 135 140  
 Tyr Leu Thr Lys Tyr Asp Cys Ser Ser Ala Asp Ile Asn Pro Ile Gly  
 145 150 155 160  
 Gly Ile Ser Lys Thr Asp Leu Arg Ala Phe Val Gln Phe Cys Ile Gln  
 165 170 175  
 Arg Phe Gln Leu Pro Ala Leu Gln Ser Ile Leu Leu Ala Pro Ala Thr  
 180 185 190  
 Ala Glu Leu Glu Pro Leu Ala Asp Gly Gln Val Ser Gln Thr Asp Glu  
 195 200 205  
 Glu Asp Met Gly Met Thr Tyr Ala Glu Leu Ser Val Tyr Gly Lys Leu  
 210 215 220  
 Arg Lys Val Ala Lys Met Gly Pro Tyr Ser Met Phe Cys Lys Leu Leu  
 225 230 235 240  
 Gly Met Trp Arg His Ile Cys Thr Pro Arg Gln Val Ala Asp Lys Val  
 245 250 255  
 Lys Arg Phe Phe Ser Lys Tyr Ser Met Asn Arg His Lys Met Thr Thr  
 260 265 270  
 Leu Thr Pro Ala Tyr His Ala Glu Asn Tyr Ser Pro Glu Asp Asn Arg  
 275 280 285  
 Phe Asp Leu Arg Pro Phe Leu Tyr Asn Thr Ser Trp Pro Trp Gln Phe  
 290 295 300  
 Arg Cys Ile Glu Asn Gln Val Leu Gln Leu Glu Arg Ala Glu Pro Gln  
 305 310 315 320  
 Ser Leu Asp

<210> 139  
 <211> 20  
 <212> PRT  
 <213> Homo sapiens

<400> 139  
 Thr His Tyr Arg Glu Ser Trp Tyr Ala Cys Arg Tyr Arg Ser Gly Ile  
 1 5 10 15  
 Pro Gly Ser Thr  
 20

<210> 140  
 <211> 20  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (4)  
 <223> Xaa equals any of the naturally occurring L-amino acids  
  
 <400> 140  
 Thr Leu Tyr Xaa Glu Cys Trp Tyr Ala Cys Arg Tyr Arg Ser Gly Ile  
 1 5 10 15

Pro Gly Ser Thr  
 20

<210> 141  
 <211> 19  
 <212> PRT  
 <213> Homo sapiens

<400> 141  
 Gly Gly Arg Ser Arg Gly Ser Lys Leu Thr Tyr Ala Cys Met Arg Arg  
 1 5 10 15

His Ser Ser

<210> 142  
 <211> 19  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (18)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 142  
 Gly Gly Arg Ser Arg Gly Ser Lys Leu Thr Tyr Ala Cys Ile Arg His  
 1 5 10 15

His Xaa Ala

<210> 143  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 143  
 Met Ala Gln Glu Thr Asn Gln Thr Pro Gly Pro Met Leu Cys Ser Thr  
 1 5 10 15

Gly Cys Gly Phe Tyr Gly Asn Pro Arg Thr Asn Gly Met Cys Ser Val  
 20 25 30

Cys Tyr Lys Glu His Leu Gln Arg Gln Gln Asn Ser Gly Arg Met Ser  
 35 40 45

Pro Met Gly Thr Ala Ser Gly Ser Asn Ser Pro Thr Ser Asp Ser Ala  
 50 55 60  
 Ser Val Gln Arg Ala Asp Thr Ser Leu Asn Asn Cys Glu Gly Ala Ala  
 65 70 75 80  
 Gly Ser Thr Ser Glu Lys Ser Arg Asn Val Pro Val Ala Ala Leu Pro  
 85 90 95  
 Val Thr Gln Gln Met Thr Glu Met Ser Ile Ser Arg Glu Asp Lys Ile  
 100 105 110  
 Thr Thr Pro Lys Thr Glu Val Ser Glu Pro Val Val Thr Gln Pro Ser  
 115 120 125  
 Pro Ser Val Ser Gln Pro Ser Thr Ser Gln Ser Glu Glu Lys Ala Pro  
 130 135 140  
 Glu Leu Pro Lys Pro Lys Lys Asn Arg Cys Phe Met Cys Arg Lys Lys  
 145 150 155 160  
 Val Gly Leu Thr Gly Phe Asp Cys Arg Cys Gly Asn Leu Phe Cys Gly  
 165 170 175  
 Leu His Arg Tyr Ser Asp Lys His Asn Cys Pro Tyr Asp Tyr Lys Ala  
 180 185 190  
 Glu Ala Ala Ala Lys Ile Arg Lys Glu Asn Pro Val Val Val Ala Glu  
 195 200 205  
 Lys Ile Gln Arg Ile  
 210

&lt;210&gt; 144

&lt;211&gt; 208

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 144

Met Ala Gln Glu Thr Asn His Ser Gln Val Pro Met Leu Cys Ser Thr  
 1 5 10 15  
 Gly Cys Gly Phe Tyr Gly Asn Pro Arg Thr Asn Gly Met Cys Ser Val  
 20 25 30  
 Cys Tyr Lys Glu His Leu Gln Arg Gln Asn Ser Ser Asn Gly Arg Ile  
 35 40 45  
 Ser Pro Pro Ala Thr Ser Val Ser Ser Leu Ser Glu Ser Leu Pro Val  
 50 55 60  
 Gln Cys Thr Asp Gly Ser Val Pro Glu Ala Gln Ser Ala Leu Asp Ser  
 65 70 75 80  
 Thr Ser Ser Ser Met Gln Pro Ser Pro Val Ser Asn Gln Ser Leu Leu  
 85 90 95

Ser Glu Ser Val Ala Ser Ser Gln Leu Asp Ser Thr Ser Val Asp Lys  
 100 105 110

Ala Val Pro Glu Thr Glu Asp Val Gln Ala Ser Val Ser Asp Thr Ala  
 115 120 125

Gln Gln Pro Ser Glu Glu Gln Ser Lys Ser Leu Glu Lys Pro Lys Gln  
 130 135 140

Lys Lys Asn Arg Cys Phe Met Cys Arg Lys Lys Val Gly Leu Thr Gly  
 145 150 155 160

Phe Glu Cys Arg Cys Gly Asn Val Tyr Cys Gly Val His Arg Tyr Ser  
 165 170 175

Asp Val His Asn Cys Ser Tyr Asn Tyr Lys Ala Asp Ala Ala Glu Lys  
 180 185 190

Ile Arg Lys Glu Asn Pro Val Val Val Glu Lys Ile Gln Lys Ile  
 195 200 205

&lt;210&gt; 145

&lt;211&gt; 69

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 145

Gln Lys Met Leu Glu Gly Ser Met Phe Val Lys Ser His His His Ser  
 1 5 10 15

Leu Ile Ser Ser Thr Gln Gly His Lys His Cys Gly Arg Pro Gln Gly  
 20 25 30

Pro Leu His Arg Lys Thr Arg Asp Leu Cys Ser Leu Val Tyr Leu Leu  
 35 40 45

Thr Leu Pro Pro Leu Leu Ser Tyr Asp Pro Ala Lys Ser Pro Ser Val  
 50 55 60

Arg Asn Thr Gln Glu  
 65

&lt;210&gt; 146

&lt;211&gt; 69

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 146

Lys Gln Met Leu Glu Gly Ser Met Leu Val Lys Ser His His His Ser  
 1 5 10 15

Leu Ile Ser Ser Thr Gln Gly His Lys His Cys Gly Arg Pro Gln Gly

[illegible]

```
<210> 147
<211> 14
<212> PRT
<213> Homo sapiens
```

<400> 147  
Asp Trp Ser Thr Pro Arg Val Phe Thr Thr Gln Asp Val Ile  
1 5 10

```
<210> 148
<211> 14
<212> PRT
<213> Homo sapiens
```

<400> 148  
Asp Trp Ser Ala His Leu Ile Phe Thr Met Gln Asp Leu Leu  
1 5 10

```
<210> 149
<211> 47
<212> PRT
<213> Homo sapiens
```

<400> 149  
Pro Glu Gly Ala Gly Gly Ser Phe Ser Ile Asp Ser Glu Glu Tyr Glu  
1 5 10 15

Ala Met Pro Val Glu Val Lys Leu Leu Pro Arg Lys Leu Gln Phe Phe  
20 25 30

Cys Asp Pro Arg Lys Arg Glu Gln Met Leu Thr Ser Pro Thr Gln  
35 40 45

```
<210> 150
<211> 47
<212> PRT
<213> Homo sapiens
```

<400> 150  
Pro Gln Gly Ala Gly Gly Ser Phe Ser Ile Asp Ser Glu Glu Tyr Glu  
1 5 10 15

Ala Met Pro Val Glu Val Lys Leu Leu Pro Arg Lys Leu Gln Phe Phe



[illegible]

Pro Ala Ala Ser Glu Pro Val Ile Arg Ser Ser Ala Val Val Asp Leu  
 1 5 10 15  
 Leu Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe Gly Ala Ser  
 20 25 30  
 Leu Phe Leu Leu Leu Ser Leu Thr Val Phe Ser Ile Val Ser Val Thr  
 35 40 45  
 Ala Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile Ser Phe Arg Ile  
 50 55 60  
 Tyr Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp Glu Gly His Pro  
 65 70 75 80  
 Phe Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile Ser Glu Glu Leu Val  
 85 90 95  
 Gln Lys Tyr Ser Asn Ser Ala Leu Gly His Val Asn Cys Thr Ile Lys  
 100 105 110  
 Glu Leu Arg Arg Leu Phe Leu Val Asp Asp Leu Val Asp Ser Leu Lys  
 115 120 125  
 Phe Ala Val Leu Met Trp Val Phe Thr Tyr Val Gly Ala Leu Phe Asn  
 130 135 140  
 Gly Leu Thr Leu Leu Ile Leu Ala Leu Ile Ser Leu Phe Ser Val Pro  
 145 150 155 160  
 Val Ile Tyr Glu Arg His Gln Ala Gln Ile Asp His Tyr Leu Gly Leu  
 165 170 175  
 Ala Asn Lys Asn Val Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile  
 180 185 190  
 Pro Gly Leu Lys Arg Lys Ala Glu  
 195 200

&lt;210&gt; 153

&lt;211&gt; 55

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 153

Asn Ser Val Thr Gln Ala Gly Val Gln Trp Arg Asn Leu Gly Ser Leu  
 1 5 10 15  
 Gln Pro Leu Pro Pro Gly Phe Lys Leu Phe Ser Cys Pro Ser Leu Leu  
 20 25 30  
 Ser Ser Trp Asp Tyr Arg Arg Pro Pro Arg Leu Ala Asn Phe Phe Val  
 35 40 45  
 Phe Leu Val Glu Met Gly Phe  
 50 55

<210> 154  
 <211> 56  
 <212> PRT  
 <213> Homo sapiens

<400> 154  
 His Ser Val Thr Gln Ala Ala Met Pro Trp Arg Asp Leu Gly Ser Leu  
 1 5 10 15  
 Gln Ala Pro Pro Gly Phe Met Pro Phe Ser Cys Leu Ser Leu Pro  
 20 25 30  
 Gly Ser Trp Asp Tyr Arg Cys Pro Pro Pro Pro Ala Asn Phe Phe  
 35 40 45  
 Val Phe Leu Val Glu Thr Gly Phe  
 50 55

<210> 155  
 <211> 92  
 <212> PRT  
 <213> Homo sapiens

<400> 155  
 Leu Leu Ser Pro Arg Leu Glu Cys Asn Gly Met Ile Leu Ala His Cys  
 1 5 10 15  
 Lys Leu Arg Leu Pro Gly Phe Lys Arg Phe Ser Cys Leu Ser Leu Pro  
 20 25 30  
 Ser Ser Trp Asp Tyr Arg His Val Pro Pro Arg Gln Val His Phe Val  
 35 40 45  
 Phe Ser Val Glu Thr Gly Phe His Arg Ala Gly Gln Ala Gly Leu Glu  
 50 55 60  
 Leu Leu Thr Ser Ser Val Pro Pro Thr Ser Ala Phe Pro Lys Cys Trp  
 65 70 75 80  
 Asp Tyr Arg Arg Asp Asp Gln Ala Trp Pro Thr Leu  
 85 90

<210> 156  
 <211> 91  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (16)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids  
 <220>  
 <221> SITE  
 <222> (69)

100

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;400&gt; 156

Val Leu Leu Pro Arg Leu Glu Cys Ser Gly Ser Ser Leu Ala His Xaa  
 1 5 10 15

Asn Leu Cys Pro Pro Val Gln Ala Ile Pro Leu Pro Gln Thr Pro Glu  
 20 25 30

Gln Pro Gly Pro Gln Ala Pro Ile Thr Thr Pro Gly Gln Pro Phe Val  
 35 40 45

Phe Leu Val Gln Thr Gly Phe His His Ala Asp Gln Ala Gly Pro Glu  
 50 55 60

Leu Leu Thr Ser Xaa Ser Ser Arg Leu Ser Leu Pro Lys Cys Trp Asp  
 65 70 75 80

Tyr Arg Arg Glu His His Ala Trp Pro Ala Leu  
 85 90

&lt;210&gt; 157

&lt;211&gt; 101

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 157

Arg Thr Tyr Ser Cys Ile His Cys Arg Ala His Leu Ala Asn His Asp  
 1 5 10 15

Glu Leu Ile Ser Lys Ser Phe Gln Gly Ser Gln Gly Arg Ala Tyr Leu  
 20 25 30

Phe Asn Ser Val Val Asn Val Gly Cys Gly Pro Ala Glu Glu Arg Val  
 35 40 45

Leu Leu Thr Gly Leu His Ala Val Ala Asp Ile Tyr Cys Glu Asn Cys  
 50 55 60

Lys Thr Thr Leu Gly Trp Lys Tyr Glu His Ala Phe Glu Ser Ser Gln  
 65 70 75 80

Lys Tyr Lys Glu Gly Lys Phe Ile Ile Glu Leu Ala His Met Ile Lys  
 85 90 95

Asp Asn Gly Trp Glu  
 100

&lt;210&gt; 158

&lt;211&gt; 101

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 158

Arg Leu Phe Ser Cys Ala Asn Cys Asp Thr Ile Leu Thr Asn Arg Ser  
 1 5 10 15

Glu Leu Ile Ser Thr Arg Phe Thr Gly Ala Thr Gly Arg Ala Phe Leu  
                   20                  25                  30  
 Phe Asn Lys Val Val Asn Leu Gln Tyr Ser Glu Val Gln Asp Arg Val  
                   35                  40                  45  
 Met Leu Thr Gly Arg His Met Val Arg Asp Val Ser Cys Lys Asn Cys  
                   50                  55                  60  
 Asn Ser Lys Leu Gly Trp Ile Tyr Glu Phe Ala Thr Glu Asp Ser Gln  
                   65                  70                  75                  80  
 Arg Tyr Lys Glu Gly Arg Val Ile Leu Glu Arg Ala Leu Val Arg Glu  
                   85                  90                  95  
 Ser Glu Gly Phe Glu  
                   100

<210> 159  
 <211> 37  
 <212> PRT  
 <213> Homo sapiens

<400> 159  
 Lys Asn Met Glu Gly Val Leu Thr Ser Ala Asp Cys Glu Lys Ile Tyr  
                   1                  5                  10                  15  
 Ser Ile Val Tyr Ile Ser Asn Arg Ala Met Ala Ser Ser Ala Gly Glu  
                   20                  25                  30  
 Phe Val Tyr Trp Lys  
                   35

<210> 160  
 <211> 37  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (20)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids  
  
 <220>  
 <221> SITE  
 <222> (24)  
 <223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 160  
 Arg Asn Met Glu Gly Val Leu Met Asp Val Asp Cys Glu Ser Val Tyr  
                   1                  5                  10                  15  
 Pro Ile Val Xaa Ala Ser Asn Xaa Gly Leu Ala Ser Ala Val Gly Glu  
                   20                  25                  30

102

Phe Leu Tyr Trp Lys  
35

<210> 161  
<211> 49  
<212> PRT  
<213> Homo sapiens

<400> 161  
Lys Ile Phe His Pro Glu Cys Gly Ala Lys Ala Val Ser Asp Arg Glu  
1 5 10 15  
Arg Arg Arg Ser Pro Gln Ala Gln Lys Thr Phe Ile Tyr Leu Leu Leu  
20 25 30  
Ala Phe Phe Met Glu Ser Glu His His Asn His Ala Ala Tyr Leu Val  
35 40 45

Asp

<210> 162  
<211> 48  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (43)  
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 162  
Arg Leu Phe Tyr Pro Glu Cys Glu Ile Arg Thr Met Gly Gly Arg Glu  
1 5 10 15  
Gln Arg Gln Ser Pro Gly Ala Gln Arg Thr Phe Phe Gln Leu Leu Leu  
20 25 30  
Cys Leu Leu Cys Gly Glu Gln Ala Asn Arg Xaa Leu Leu Gln Ala Asp  
35 40 45

<210> 163  
<211> 220  
<212> PRT  
<213> Homo sapiens

<400> 163  
Pro Phe Pro Lys Gly Ile Cys Thr Lys Cys Lys Pro Gln Val Val Thr  
1 5 10 15  
Leu Asn Arg Gln Lys Phe Arg His Val Asp Asn Ile Gln Ile Glu Asn  
20 25 30

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Glu | Leu | Val | Asn | Gln | Phe | Leu | Asp | Tyr | Trp | Arg | Leu | Ser | Gly | His |
|     |     | 35  |     |     |     |     |     | 40  |     |     |     |     | 45  |     |     |
| Gln | Arg | Val | Gly | Phe | Leu | Ile | Gly | Gln | Tyr | Gln | Pro | His | Leu | Glu | Val |
|     |     | 50  |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Pro | Leu | Gly | Ile | Lys | Ala | Thr | Val | Ala | Ala | Ile | Tyr | Glu | Pro | Pro | Gln |
|     |     | 65  |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| His | Cys | Arg | Glu | Asp | Gly | Ile | Glu | Phe | Leu | Glu | Asp | Lys | Asn | Gln | Lys |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Thr | Ile | Asp | Asn | Leu | Leu | Glu | Met | Leu | Gly | Leu | Gln | Arg | Val | Gly | Trp |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Ile | Phe | Thr | Asp | Cys | Trp | Thr | Ala | Asn | Ser | Ala | Glu | Gly | Thr | Val | His |
|     |     |     | 115 |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Tyr | Thr | Arg | His | Lys | Asp | Ser | Phe | Phe | Leu | Ser | Ala | Glu | Glu | Cys | Ile |
|     |     | 130 |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Thr | Ala | Ala | Met | Leu | Gln | Asn | Gln | His | Pro | Asn | Ile | Thr | Glu | Tyr | Ser |
|     |     |     | 145 |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Met | Asp | Arg | His | Tyr | Gly | Ser | Lys | Phe | Val | Thr | Val | Val | Ala | Ser | Gly |
|     |     |     | 165 |     |     |     |     | 170 |     |     |     |     |     | 175 |     |
| Asp | Glu | Ser | Met | His | Val | Asn | Phe | His | Gly | Tyr | Gln | Val | Ser | Asn | Gln |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Cys | Ala | Ala | Met | Val | Glu | Ala | Asp | Ile | Leu | Cys | Pro | Thr | Leu | Tyr | Thr |
|     |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Pro | Glu | Leu | Ala | Tyr | Val | Arg | Glu | Thr | Pro | Leu | Ser |     |     |     |     |
|     |     | 210 |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |

&lt;210&gt; 164

 $\langle 211 \rangle$  220

&lt;212&gt; PRT

<213> Homo sapiens

<220>

&lt;221&gt; SITE

<222> (5)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 164

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Trp | Pro | Asn | Xaa | Leu | Cys | Thr | Lys | Cys | Gln | Pro | Ser | Ala | Ile | Thr |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Leu | Asn | Arg | Gln | Lys | Tyr | Arg | His | Val | Asp | Asn | Ile | Met | Phe | Glu | Asn |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| His | Thr | Val | Ala | Asp | Arg | Phe | Leu | Asp | Phe | Trp | Arg | Lys | Thr | Gly | Asn |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

104

Gln His Phe Gly Tyr Leu Tyr Gly Arg Tyr Thr Glu His Lys Asp Ile  
 50 55 60  
 Pro Leu Gly Ile Arg Ala Glu Val Ala Ala Ile Tyr Glu Pro Pro Gln  
 65 70 75 80  
 Ile Gly Thr Gln Asn Ser Leu Glu Leu Leu Glu Asp Pro Lys Ala Gln  
 85 90 95  
 Val Val Asp Glu Ile Ala Ala Lys Leu Gly Leu Arg Lys Val Gly Trp  
 100 105 110  
 Ile Phe Thr Asp Leu Val Ser Glu Asp Thr Arg Lys Gly Thr Val Arg  
 115 120 125  
 Tyr Ser Arg Asn Lys Asp Thr Tyr Phe Leu Ser Ser Glu Glu Cys Ile  
 130 135 140  
 Thr Ala Gly Asp Phe Gln Asn Lys His Pro Asn Met Cys Arg Leu Ser  
 145 150 155 160  
 Pro Asp Gly His Phe Gly Ser Lys Phe Val Thr Ala Val Ala Thr Gly  
 165 170 175  
 Gly Pro Asp Asn Gln Val His Phe Glu Gly Tyr Gln Val Ser Asn Gln  
 180 185 190  
 Cys Met Ala Leu Val Arg Asp Glu Cys Leu Leu Pro Cys Lys Asp Ala  
 195 200 205  
 Pro Glu Leu Ala Thr Pro Arg Ser Leu Ala Val Ser  
 210 215 220

&lt;210&gt; 165

&lt;211&gt; 215

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 165

Pro Glu Leu Ala Tyr Val Arg Glu Thr Pro Leu Ser Glu Glu His Tyr  
 1 5 10 15  
 Ile Thr Asp Val Gln Phe Ser Met Lys Asn Glu Tyr Gly Ala Glu Val  
 20 25 30  
 Met Lys Asn Gly Arg Pro Leu Pro Val Glu Tyr Leu Leu Val Asp Val  
 35 40 45  
 Pro Ala Gly Met Pro Lys Glu Pro His Tyr Thr Phe His Val Gly Thr  
 50 55 60  
 Ser Asn Lys Ser Lys Ser Ala Lys Phe Asn Val Glu Asn Arg Gln Ala  
 65 70 75 80  
 Ile Gly Gln Leu Gln Gly Gly Ala Asn Leu Ile Gln Tyr Ser Ser Glu  
 85 90 95



Phe Ser Thr Asn Gln Phe Leu Glu Gln Ala Thr Asn Phe His Phe Leu  
                   100                                  105                                  110  
 Leu Tyr Leu Val Thr Asn Asp Gln Val Gln Ile Ser Asp Glu Trp Met  
           115                                  120                                  125  
 Lys Arg Leu Cys Asp Ala Val Lys Ala Gln Asp Arg Gly Thr Ala Met  
           130                                  135                                  140  
 Glu Trp Ala Gln Glu Cys Glu Asp Trp His Gln Leu Met Ala Leu Ala  
   145                                  150                                  155                                  160  
 His Ala Asn Gly Gly Ser Gly Asn Asp Asp Ile Pro Val Ile Pro Asn  
                   165                                  170                                  175  
 Gly Asp Pro Phe Ser Gly Ser Ser Ser Gly Gly Ser Arg Gly Ala Val  
                   180                                  185                                  190  
 Trp Ser Cys Gly His Cys Thr Phe Gln Asn Glu Ala Gly Arg Gln Asp  
           195                                  200                                  205  
 Cys Ser Met Cys Gly Leu Pro  
       210                                  215

&lt;210&gt; 166

&lt;211&gt; 200

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (13)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (90)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 166

Pro Gly Ala Gly Tyr Ala Lys Glu Ser Ser Ser Glu Xaa Tyr Val Pro  
   1                                  5                                  10                                  15  
 Asp Val Phe Tyr Lys Asp Val Asp Lys Phe Gly Asn Glu Ile Thr Gln  
           20                                  25                                  30  
 Leu Ala Arg Pro Leu Pro Val Glu Tyr Leu Ile Ile Asp Ile Thr Thr  
           35                                  40                                  45  
 Thr Phe Pro Lys Asp Pro Val Tyr Thr Phe Ser Ile Ser Gln Asn Pro  
   50                                  55                                  60  
 Phe Pro Ile Glu Asn Arg Asp Val Leu Gly Glu Thr Gln Asp Phe His  
   65                                  70                                  75                                  80  
 Ser Leu Ala Thr Tyr Leu Ser Gln Asn Xaa Ser Ser Val Phe Leu Asp  
           85                                  90                                  95

Thr Ile Ser Asp Phe His Leu Leu Phe Leu Val Thr Asn Glu Val  
 100 105 110  
 Met Pro Leu Gln Asp Ser Ile Ser Leu Leu Leu Glu Ala Val Arg Thr  
 115 120 125  
 Arg Asn Glu Glu Leu Ala Gln Thr Trp Lys Arg Ser Glu Gln Trp Ala  
 130 135 140  
 Thr Ile Glu Gln Leu Cys Ser Thr Val Gly Gly Gln Leu Pro Gly Leu  
 145 150 155 160  
 His Glu Tyr Gly Ala Val Gly Gly Ser Thr His Thr Ala Thr Ala Ala  
 165 170 175  
 Met Trp Ala Cys Gln His Cys Thr Phe Met Asn Gln Pro Gly Thr Gly  
 180 185 190  
 His Cys Glu Met Cys Ser Leu Pro  
 195 200

&lt;210&gt; 167

&lt;211&gt; 289

&lt;212&gt; FRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 167

Pro Pro Gln Tyr Val Ala Gly Pro Ala Arg Tyr Glu Glu Ala Arg Ile  
 1 5 10 15  
 Thr Ile Leu Thr Gly Val Pro Val Asp Thr Val Asp Arg Gln Ser Lys  
 20 25 30  
 Leu Val Arg Leu Ser Asp Gly Arg Lys Phe Pro Tyr Asp Gln Leu Leu  
 35 40 45  
 Leu Ala Thr Gly Ala Arg Pro Arg Pro Phe Ala Gly Val Ser Gly Asp  
 50 55 60  
 Pro Gly Leu Gly Arg Ile Cys Met Leu Arg Asn His Gly Asp Ala Val  
 65 70 75 80  
 Ala Ile His Glu Ala Leu Ala Pro Gly Lys Ser Leu Ala Val Val Gly  
 85 90 95  
 Gly Gly Phe Ile Gly Leu Glu Ile Ala Ala Thr Ala Arg Lys Leu Gly  
 100 105 110  
 Ala Glu Val Leu Leu Ile Glu Ser Ser Pro Arg Val Leu Ser Arg Gly  
 115 120 125  
 Val Pro Ala Glu Ile Ala Lys Val Val Thr Glu Arg His Arg Gln Glu  
 130 135 140  
 Gly Val Asn Ile Leu Cys Gly Glu Gln Ile Thr Ser Ile Ala Thr Glu  
 145 150 155 160

Asn Asp Lys Val Gln Ile Leu Phe Ala Asn Gly Thr Thr Arg Leu Ala  
 165 170 175  
 Asp Ile Val Val Val Gly Ile Gly Val Ile Pro Asn Val Glu Leu Ala  
 180 185 190  
 Glu Ala Ala Gly Leu Leu Ile Asp Asn Gly Ile Ala Val Asp Ala Thr  
 195 200 205  
 Leu Arg Thr Ser Asp Pro Asp Ile Phe Ala Ala Gly Asp Cys Cys Ser  
 210 215 220  
 Phe Pro Leu Ser His Tyr Arg Glu Arg Arg Val Arg Leu Glu Ala Trp  
 225 230 235 240  
 Arg Asn Ala Gln Asp Gln Gly Met Leu Val Ala Ala Asn Leu Leu Gly  
 245 250 255  
 Arg Gly Leu Ala Ile Ala Ser Val Pro Trp Phe Trp Ser Asp Gln Tyr  
 260 265 270  
 Glu Leu Thr Leu Gln Ile Ala Gly Leu Ser Asp Gly Ala Ala Thr Thr  
 275 280 285  
 Val

<210> 168  
 <211> 299  
 <212> PRT  
 <213> Homo sapiens  
 <400> 168  
 Pro Pro Ser Phe Tyr Val Ser Ala Gln Asp Leu Pro His Ile Glu Asn  
 1 5 10 15  
 Gly Gly Val Ala Val Leu Thr Gly Lys Lys Val Val Gln Leu Asp Val  
 20 25 30  
 Arg Asp Asn Met Val Lys Leu Asn Asp Gly Ser Gln Ile Thr Tyr Glu  
 35 40 45  
 Lys Cys Leu Ile Ala Thr Gly Gly Thr Pro Arg Ser Leu Ser Ala Ile  
 50 55 60  
 Asp Arg Ala Gly Ala Glu Val Lys Ser Arg Thr Thr Leu Phe Arg Lys  
 65 70 75 80  
 Ile Gly Asp Phe Arg Ser Leu Glu Lys Ile Ser Arg Glu Val Lys Ser  
 85 90 95  
 Ile Thr Ile Ile Gly Gly Gly Phe Leu Gly Ser Glu Leu Ala Cys Ala  
 100 105 110  
 Leu Gly Arg Lys Ala Arg Ala Leu Gly Thr Glu Val Ile Gln Leu Phe  
 115 120 125

Pro Glu Lys Gly Asn Met Gly Lys Ile Leu Pro Glu Tyr Leu Ser Asn  
 130 135 140  
 Trp Thr Met Glu Lys Val Arg Arg Glu Gly Val Lys Val Met Pro Asn  
 145 150 155 160  
 Ala Ile Val Gln Ser Val Gly Val Ser Ser Gly Lys Leu Leu Ile Lys  
 165 170 175  
 Leu Lys Asp Gly Arg Lys Val Glu Thr Asp His Ile Val Ala Ala Val  
 180 185 190  
 Gly Leu Glu Pro Asn Val Glu Leu Ala Lys Thr Gly Gly Leu Glu Ile  
 195 200 205  
 Asp Ser Asp Phe Gly Gly Phe Arg Val Asn Ala Glu Leu Gln Ala Arg  
 210 215 220  
 Ser Asn Ile Trp Val Ala Gly Asp Ala Ala Cys Phe Tyr Asp Ile Lys  
 225 230 235 240  
 Leu Gly Arg Arg Arg Val Glu His His Asp His Ala Val Val Ser Gly  
 245 250 255  
 Arg Leu Ala Gly Glu Asn Met Thr Gly Ala Ala Lys Pro Tyr Trp His  
 260 265 270  
 Gln Ser Met Phe Trp Ser Asp Leu Gly Pro Asp Val Gly Tyr Glu Ala  
 275 280 285  
 Ile Gly Leu Val Asp Ser Ser Leu Pro Thr Val  
 290 295  
  
 <210> 169  
 <211> 137  
 <212> PRT  
 <213> Homo sapiens  
  
 <400> 169  
 Met Asn Thr Glu Met Tyr Gln Thr Pro Met Glu Val Ala Val Tyr Gln  
 1 5 10 15  
 Leu His Asn Phe Ser Thr Ser Phe Phe Ser Ser Leu Leu Gly Gly Asp  
 20 25 30  
 Val Val Ser Val Lys Leu Asp Asn Ser Ala Ser Gly Ala Ser Val Val  
 35 40 45  
 Ala Leu Asp Asn Lys Ile Glu Gln Ala Met Asp Leu Val Lys Asn His  
 50 55 60  
 Leu Met Tyr Ala Val Arg Glu Glu Val Glu Val Leu Lys Glu Gln Ile  
 65 70 75 80  
 Arg Glu Leu Leu Glu Lys Asn Ser Gln Leu Glu Arg Glu Asn Thr Leu  
 85 90 95

109

Leu Lys Thr Leu Ala Ser Pro Glu Gln Leu Glu Lys Phe Gln Ser Arg  
 100 105 110

Leu Ser Pro Glu Glu Pro Ala Pro Glu Ala Pro Glu Thr Pro Glu Thr  
 115 120 125

Pro Glu Ala Pro Gly Gly Ser Ala Val  
 130 135

&lt;210&gt; 170

&lt;211&gt; 134

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 170

Met Asn Thr Glu Met Tyr Gln Thr Pro Met Glu Val Ala Val Tyr Gln  
 1 5 10 15

Leu His Asn Phe Ser Ile Ser Phe Phe Ser Ser Leu Leu Gly Gly Asp  
 20 25 30

Val Val Ser Val Lys Leu Asp Asn Ser Ala Ser Gly Ala Ser Val Val  
 35 40 45

Ala Ile Asp Asn Lys Ile Glu Gln Ala Met Asp Leu Val Lys Asn His  
 50 55 60

Leu Met Tyr Ala Val Arg Glu Glu Val Glu Ile Leu Lys Glu Gln Ile  
 65 70 75 80

Arg Glu Leu Val Glu Lys Asn Ser Gln Leu Glu Arg Glu Asn Thr Leu  
 85 90 95

Leu Lys Thr Leu Ala Ser Pro Glu Gln Leu Glu Lys Phe Gln Ser Cys  
 100 105 110

Leu Ser Pro Glu Glu Pro Ala Pro Glu Ser Pro Gln Val Pro Glu Ala  
 115 120 125

Pro Gly Gly Ser Ala Val  
 130

&lt;210&gt; 171

&lt;211&gt; 104

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 171

Ile Tyr Ala Ala Asn Arg His Met Lys Lys Cys Ser Ser Ser Leu Ala  
 1 5 10 15

Ile Arg Glu Met Gln Ile Lys Thr Thr Met Arg Tyr His Leu Thr Pro  
 20 25 30

Val Arg Met Val Ile Ile Arg Lys Ser Gly Asn Asp Arg Cys Trp Arg

Gly Cys Gly Glu Ile Gly Thr Leu Leu His Cys Trp Trp Asp Cys Lys  
           50               55               60  
 Leu Val Gln Pro Leu Trp Lys Thr Val Trp Arg Phe Leu Arg Asp Leu  
         65               70               75               80  
 Gln Leu Glu Ile Pro Phe Asp Pro Ala Ile Pro Leu Leu Gly Ile Tyr  
             85                         90                 95  
 Pro Lys Asp Tyr Lys Ser Cys Cys  
           100

```
<210> 172
<211> 107
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (39)
<223> Xaa equals any of the twenty naturally occurring L-amino acids
```

```
<220>
<221> SITE
<222> (51)
<223> Xaa equals any of the twenty naturally occurring L-amino acids

<220>
<221> SITE
<222> (62)
<223> Xaa equals any of the twenty naturally occurring L-amino acids
```

```

<400> 172
Leu Tyr Met Asn Leu Asn His Ile Ser Lys Trp Leu Thr Val Ile Lys
 1 5 10 15
Val Val Thr Arg Lys Met Gln Ile Lys Thr Thr Leu Gln Tyr His Cys
 20 25 30
Thr Pro Thr Ser Thr Val Xaa Lys Lys Lys Lys Ala Glu Asn Ile Lys
 35 40 45
Tyr Trp Xaa Gln Cys Gly Ala Asn Arg Thr Leu Phe Gln Xaa Trp Gln
 50 55 60
Val Cys Lys Val Ala Gln Thr Leu Trp Lys Pro Val Trp His Tyr Thr
 65 70 75 80
Val Leu Asn Leu Asn Thr Arg Ile Val Tyr Asp Pro Gly Met Pro Leu
 85 90 95
Leu Gly Thr Asn Asn Asn Ala Tyr Met Cys Cys
 100 105

```

111

<210> 173  
 <211> 336  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (13)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (43)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (121)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (138)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (288)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 173  
 Glu Pro Ala Cys Ala His Ala Leu Glu Ala Leu Ala Xaa Leu Leu Arg  
 1 5 10 15  
 Pro Arg Tyr Leu Asp Leu Leu Val Ser Ser Pro Ala Phe Pro Gln Pro  
 20 25 30  
 Val Gly Ser Leu Ser Pro Glu Pro Met Pro Xaa Glu Arg Phe Gly Arg  
 35 40 45  
 Arg Phe Pro Leu Ala Pro Gly Arg Arg Leu Glu Glu Tyr Gly Ala Phe  
 50 55 60  
 Tyr Val Gly Gly Ser Lys Ala Ser Pro Asp Pro Asp Leu Asp Pro Asp  
 65 70 75 80  
 Leu Ser Arg Leu Leu Leu Gly Trp Ala Pro Gly Gln Pro Phe Ser Ser  
 85 90 95  
 Cys Cys Pro Asp Thr Gly Lys Thr Gln Asp Glu Gly Ala Arg Ala Gly  
 100 105 110  
 Gly Leu Arg Ala Arg Arg Tyr Xaa Val Glu Arg Ala Arg Asp Ala  
 115 120 125  
 Arg Val Val Gly Leu Leu Ala Gly Thr Xaa Gly Val Ala Gln His Arg  
 130 135 140

112

Glu Ala Leu Ala His Leu Arg Asn Leu Thr Gln Ala Ala Gly Lys Arg  
145 150 155 160

Ser Tyr Val Leu Ala Leu Gly Arg Pro Thr Pro Ala Lys Leu Ala Asn  
165 170 175

Phe Pro Glu Val Asp Val Phe Val Leu Leu Ala Cys Pro Leu Gly Ala  
180 185 190

Leu Ala Pro Gln Leu Ser Gly Ser Phe Phe Gln Pro Ile Leu Ala Pro  
195 200 205

Cys Glu Leu Glu Ala Ala Cys Asn Pro Ala Trp Pro Pro Gly Leu  
210 215 220

Ala Pro His Leu Thr His Tyr Ala Asp Leu Leu Pro Gly Ser Pro Phe  
225 230 235 240

His Val Ala Leu Pro Pro Pro Glu Ser Glu Leu Trp Glu Thr Pro Asp  
245 250 255

Val Ser Leu Ile Thr Gly Asp Leu Arg Pro Pro Pro Ala Trp Lys Ser  
260 265 270

Ser Asn Asp His Gly Ser Leu Ala Leu Asn Pro Arg Pro Gln Leu Xaa  
275 280 285

Leu Ala Glu Ser Ser Pro Ala Val Ser Phe Leu Ser Ser Arg Ser Trp  
290 295 300

Gln Gly Leu Glu Pro Arg Leu Gly Gln Thr Pro Val Thr Glu Ala Val  
305 310 315 320

Ser Gly Arg Arg Gly Ile Ala Ile Ala Tyr Glu Asp Glu Gly Ser Gly  
325 330 335

<210> 174

<211> 336

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (161)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 174

Glu Pro Ala Cys Ala His Ala Leu Glu Ala Leu Ala Thr Leu Leu Arg  
1 5 10 15

Pro Arg Tyr Leu Asp Leu Leu Val Ser Ser Pro Ala Phe Pro Gln Pro  
20 25 30

Val Gly Ser Leu Ser Pro Glu Pro Met Pro Leu Glu Arg Phe Gly Arg



|                                                                 |                                     |     |
|-----------------------------------------------------------------|-------------------------------------|-----|
| 35                                                              | 40                                  | 45  |
| Arg Phe Pro Leu Ala Pro Gly                                     | Arg Arg Leu Glu Glu Tyr Gly Ala Phe |     |
| 50                                                              | 55                                  | 60  |
| Tyr Val Gly Gly Ser Lys Ala Ser Pro Asp                         | Pro Asp Leu Asp Pro Asp             |     |
| 65                                                              | 70                                  | 75  |
| Leu Ser Arg Leu Leu Leu Gly Trp Ala Pro Gly Gln Pro Phe Ser Ser |                                     |     |
|                                                                 | 85                                  | 90  |
| Cys Cys Pro Asp Thr Gly Lys Thr Gln Asp Glu Gly Ala Arg Ala Gly |                                     |     |
|                                                                 | 100                                 | 105 |
| Arg Leu Arg Ala Arg Arg Arg Tyr Leu Val Glu Arg Ala Arg Asp Ala |                                     |     |
|                                                                 | 115                                 | 120 |
| Arg Val Val Gly Leu Leu Ala Gly Thr Leu Gly Val Ala Gln His Arg |                                     |     |
|                                                                 | 130                                 | 135 |
| Glu Ala Leu Ala His Leu Arg Asn Leu Thr Gln Ala Ala Gly Lys Arg |                                     |     |
|                                                                 | 145                                 | 150 |
| Xaa Tyr Val Leu Ala Leu Gly Arg Pro Thr Pro Ala Lys Leu Ala Asn |                                     |     |
|                                                                 | 165                                 | 170 |
| Phe Pro Glu Val Asp Val Phe Val Leu Leu Ala Cys Pro Leu Gly Ala |                                     |     |
|                                                                 | 180                                 | 185 |
| Leu Ala Pro Gln Leu Ser Gly Ser Phe Phe Gln Pro Ile Leu Ala Pro |                                     |     |
|                                                                 | 195                                 | 200 |
| Cys Glu Leu Glu Ala Ala Cys Asn Pro Ala Trp Pro Pro Pro Gly Leu |                                     |     |
|                                                                 | 210                                 | 215 |
| Ala Pro His Leu Thr His Tyr Ala Asp Leu Leu Pro Gly Ser Pro Phe |                                     |     |
|                                                                 | 225                                 | 230 |
| His Val Ala Leu Pro Pro Pro Glu Ser Glu Leu Trp Glu Thr Pro Asp |                                     |     |
|                                                                 | 245                                 | 250 |
| Val Ser Leu Ile Thr Gly Asp Leu Arg Pro Pro Pro Ala Trp Lys Ser |                                     |     |
|                                                                 | 260                                 | 265 |
| Ser Asn Asp His Gly Ser Leu Ala Leu Thr Pro Arg Pro Gln Leu Glu |                                     |     |
|                                                                 | 275                                 | 280 |
| Leu Ala Glu Ser Ser Pro Ala Ala Ser Phe Leu Ser Ser Arg Ser Trp |                                     |     |
|                                                                 | 290                                 | 295 |
| Gln Gly Leu Glu Pro Arg Leu Gly Gln Thr Pro Val Thr Glu Ala Val |                                     |     |
|                                                                 | 305                                 | 310 |
| Ser Gly Arg Arg Gly Ile Ala Ile Ala Tyr Glu Asp Glu Gly Ser Gly |                                     |     |
|                                                                 | 325                                 | 330 |
|                                                                 |                                     | 335 |

114

&lt;210&gt; 175

&lt;211&gt; 80

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 175

```

Gly His Leu Pro Asp Ala Glu Leu Val Ala Phe Leu Lys Arg Cys Ile
 1 5 10 15
Val Gly Leu Gln Pro Asn Gly Thr Ile Val Val Lys Glu Asn Asn Thr
 20 25 30
Pro Thr Asp Thr Asp Asp Phe Asp Glu Thr Asp Ser Ser Val Thr Arg
 35 40 45
Ser Asp Ala Lys Phe Arg Gln Ile Phe Glu Glu Ala Gly Leu Lys Leu
 50 55 60
Ile Ala Ser Glu Arg Gln Arg Gly Leu Pro Arg Glu Leu Tyr Pro Val
 65 70 75 80

```

&lt;210&gt; 176

&lt;211&gt; 79

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 176

```

Gly His Leu Thr Asp Gln His Leu Ala Glu Phe Leu Arg Arg Cys Lys
 1 5 10 15
Gly Ser Leu Arg Pro Asn Gly Ile Ile Val Ile Lys Asp Asn Met Ala
 20 25 30
Gln Glu Gly Val Ile Leu Asp Asp Val Asp Ser Ser Val Cys Arg Asp
 35 40 45
Leu Asp Val Val Arg Arg Ile Ile Cys Ser Ala Gly Leu Ser Leu Leu
 50 55 60
Ala Glu Glu Arg Gln Glu Asn Leu Pro Asp Glu Ile Tyr His Val
 65 70 75

```

&lt;210&gt; 177

&lt;211&gt; 224

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 177

```

Met Glu Ser Gly Ala Tyr Gly Ala Ala Lys Ala Gly Gly Ser Phe Asp
 1 5 10 15

```

Leu Arg Arg Phe Leu Thr Gln Pro Gln Val Val Ala Arg Ala Val Cys  
                   20                                  25                                  30  
 Leu Val Phe Ala Leu Ile Val Phe Ser Cys Ile Tyr Gly Glu Gly Tyr  
                   35                                  40                                  45  
 Ser Asn Ala His Glu Ser Lys Gln Met Tyr Cys Val Phe Asn Arg Asn  
                   50                                  55                                  60  
 Glu Asp Ala Cys Arg Tyr Gly Ser Ala Ile Gly Val Leu Ala Phe Leu  
                   65                                  70                                  75                                  80  
 Ala Ser Ala Phe Phe Leu Val Val Asp Ala Tyr Phe Pro Gln Ile Ser  
                   85                                  90                                  95  
 Asn Ala Thr Asp Arg Lys Tyr Leu Val Ile Gly Asp Leu Leu Phe Ser  
                   100                                  105                                  110  
 Ala Leu Trp Thr Phe Leu Trp Phe Val Gly Phe Cys Phe Leu Thr Asn  
                   115                                  120                                  125  
 Gln Trp Ala Val Thr Asn Pro Lys Asp Val Leu Val Gly Ala Asp Ser  
                   130                                  135                                  140  
 Val Arg Ala Ala Ile Thr Phe Ser Phe Phe Ser Ile Phe Ser Trp Gly  
                   145                                  150                                  155                                  160  
 Val Leu Ala Ser Leu Ala Tyr Gln Arg Tyr Lys Ala Gly Val Asp Asp  
                   165                                  170                                  175  
 Phe Ile Gln Asn Tyr Val Asp Pro Thr Pro Asp Pro Asn Thr Ala Tyr  
                   180                                  185                                  190  
 Ala Ser Tyr Pro Gly Ala Ser Val Asp Asn Tyr Gln Gln Pro Pro Phe  
                   195                                  200                                  205  
 Thr Gln Asn Ala Glu Thr Thr Glu Gly Tyr Gln Pro Pro Pro Val Tyr  
                   210                                  215                                  220

&lt;210&gt; 178

&lt;211&gt; 224

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (137)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 178

Met Glu Ser Gly Ala Tyr Gly Ala Ala Lys Ala Gly Gly Ser Phe Asp  
                   1                                  5                                  10                                  15

Leu Arg Arg Phe Leu Thr Gln Pro Gln Val Val Ala Arg Ala Val Cys

|                                                                 |                                 |                         |             |  |    |
|-----------------------------------------------------------------|---------------------------------|-------------------------|-------------|--|----|
|                                                                 | 20                              |                         | 25          |  | 30 |
| Leu Val Phe                                                     | Ala Leu Ile Val                 | Phe Ser Cys Ile Tyr Gly | Glu Gly Tyr |  |    |
| 35                                                              |                                 | 40                      | 45          |  |    |
| Ser Asn Ala His                                                 | Glu Ser Lys Gln Met Tyr Cys Val | Phe Asn Arg Asn         |             |  |    |
| 50                                                              | 55                              | 60                      |             |  |    |
| Glu Asp Ala Cys Arg Tyr Gly Ser Ala Ile Gly Val Leu Ala Phe Leu |                                 |                         |             |  |    |
| 65                                                              | 70                              | 75                      | 80          |  |    |
| Ala Ser Ala Phe Phe Leu Val Val Asp Ala Tyr Phe Pro Gln Ile Ser |                                 |                         |             |  |    |
|                                                                 | 85                              | 90                      | 95          |  |    |
| Asn Ala Thr Asp Arg Lys Tyr Leu Val Ile Gly Asp Leu Leu Phe Ser |                                 |                         |             |  |    |
| 100                                                             | 105                             | 110                     |             |  |    |
| Ala Leu Trp Thr Phe Leu Trp Phe Val Gly Phe Cys Phe Leu Thr Asn |                                 |                         |             |  |    |
| 115                                                             | 120                             | 125                     |             |  |    |
| Gln Trp Ala Val Thr Asn Pro Lys Xaa Val Leu Val Gly Ala Asp Ser |                                 |                         |             |  |    |
| 130                                                             | 135                             | 140                     |             |  |    |
| Val Arg Ala Ala Ile Thr Phe Ser Phe Phe Ser Ile Phe Ser Trp Gly |                                 |                         |             |  |    |
| 145                                                             | 150                             | 155                     | 160         |  |    |
| Val Leu Ala Ser Leu Ala Tyr Gln Arg Tyr Lys Ala Gly Val Asp Asp |                                 |                         |             |  |    |
|                                                                 | 165                             | 170                     | 175         |  |    |
| Phe Ile Gln Asn Tyr Val Asp Pro Thr Pro Asp Pro Asn Thr Ala Tyr |                                 |                         |             |  |    |
| 180                                                             | 185                             | 190                     |             |  |    |
| Ala Ser Tyr Pro Gly Ala Ser Val Asp Asn Tyr Gln Gln Pro Pro Phe |                                 |                         |             |  |    |
| 195                                                             | 200                             | 205                     |             |  |    |
| Thr Gln Asn Ala Glu Thr Thr Glu Gly Tyr Gln Pro Pro Pro Val Tyr |                                 |                         |             |  |    |
| 210                                                             | 215                             | 220                     |             |  |    |

<210> 179  
 <211> 188  
 <212> PRT  
 <213> Homo sapiens

<400> 179  
 Lys Gly Met Leu Val Met Asp Cys His Arg Thr His Leu Ser Glu Gly  
 1 5 10 15  
 Val Leu Ala Met Leu Ser Ala Ser Ser Thr Leu Pro Ala Val Val Pro  
 20 25 30  
 Ala Gly Cys Ser Ser Lys Ile Gln Pro Leu Asp Val Cys Ile Lys Arg  
 35 40 45  
 Thr Val Lys Asn Phe Leu His Lys Lys Trp Lys Glu Gln Ala Arg Glu

50                      55                      60  
 Met Ala Asp Thr Ala Cys Asp Ser Asp Val Leu Leu Gln Leu Val Leu  
 65                      70                      75                      80  
 Val Trp Leu Gly Glu Val Leu Gly Val Ile Gly Asp Cys Pro Glu Leu  
 85                      90                      95  
 Val Gln Arg Ser Phe Leu Val Ala Ser Val Leu Pro Gly Pro Asp Gly  
 100                      105                      110  
 Asn Ile Asn Ser Pro Thr Arg Asn Ala Asp Met Gln Glu Glu Leu Ile  
 115                      120                      125  
 Ala Ser Leu Glu Glu Gln Leu Lys Leu Ser Gly Glu His Ser Glu Ser  
 130                      135                      140  
 Ser Thr Pro Arg Pro Arg Ser Ser Pro Glu Glu Thr Ile Glu Pro Glu  
 145                      150                      155                      160  
 Ser Leu His Gln Leu Phe Glu Gly Glu Ser Glu Thr Glu Ser Phe Tyr  
 165                      170                      175  
 Gly Phe Glu Glu Ala Asp Leu Asp Leu Met Glu Ile  
 180                      185

&lt;210&gt; 180

&lt;211&gt; 188

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (146)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 180

Lys Gly Met Leu Val Met Asp Cys His Arg Thr His Leu Ser Glu Glu  
 1                      5                      10                      15

Val Leu Ala Met Leu Ser Ala Ser Ser Thr Leu Pro Ala Val Val Pro  
 20                      25                      30

Ala Gly Cys Ser Ser Lys Ile Gln Pro Leu Asp Val Cys Ile Lys Arg  
 35                      40                      45

Thr Val Lys Asn Phe Leu His Lys Lys Trp Lys Glu Gln Ala Arg Glu  
 50                      55                      60

Met Ala Asp Thr Ala Cys Asp Ser Asp Val Leu Leu Gln Leu Val Leu  
 65                      70                      75                      80

Val Trp Leu Gly Glu Val Leu Gly Val Ile Gly Asp Cys Pro Glu Leu  
 85                      90                      95

Val Gln Arg Ser Phe Leu Val Ala Ser Val Leu Pro Gly Pro Asp Gly  
 100                      105                      110

118

Asn Ile Asn Ser Pro Thr Arg Asn Ala Asp Met Gln Glu Glu Leu Ile  
 115 120 125  
 Ala Ser Leu Glu Glu Gln Leu Lys Leu Ser Gly Glu His Ser Glu Ser  
 130 135 140  
 Ser Xaa Pro Arg Pro Arg Ser Ser Pro Glu Glu Thr Ile Glu Pro Glu  
 145 150 155 160  
 Ser Leu His Gln Leu Phe Glu Gly Glu Ser Glu Thr Glu Ser Phe Tyr  
 165 170 175  
 Gly Phe Glu Glu Ala Asp Leu Asp Leu Met Glu Ile  
 180 185

<210> 181  
 <211> 22  
 <212> PRT  
 <213> Homo sapiens

<400> 181  
 Met Glu Leu Trp Ser Thr Arg Val Trp Gln Lys His Thr Ala Cys Gln  
 1 5 10 15  
 Arg Ser Lys Gly Met Leu  
 20

<210> 182  
 <211> 22  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (18)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 182  
 Val Glu Leu Trp Ser Thr Arg Val Trp Gln Lys His Thr Ala Cys Gln  
 1 5 10 15  
 Arg Xaa Lys Ala Cys Leu  
 20

<210> 183  
 <211> 80  
 <212> PRT  
 <213> Homo sapiens

<400> 183  
 His Asp Phe Gly Ser Leu Gln Pro Leu Pro Pro Gly Phe Lys Arg Phe  
 1 5 10 15  
 Ser Cys Leu Ser Leu Pro Arg Ser Trp Asp Tyr Arg His Pro Pro Pro

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | 20  |     | 25  |     | 30  |     |     |     |     |     |     |     |     |     |     |
| Arg | Pro | Ala | Asn | Phe | Glu | Phe | Leu | Val | Glu | Thr | Gly | Phe | Leu | His | Val |
|     | 35  |     |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Gly | Gln | Ala | Gly | Leu | Glu | Leu | Leu | Thr | Ser | Gly | Asp | Leu | Pro | Ala | Ser |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Ala | Ser | Gln | Ser | Ala | Arg | Ile | Thr | Gly | Val | Ser | His | Arg | Ala | Arg | Pro |
|     | 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

&lt;210&gt; 184

&lt;211&gt; 80

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (50)

&lt;223&gt; Xaa equals any of the twenty naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (75)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 184

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Asp | Leu | Gly | Ser | Pro | Gln | Pro | Leu | Pro | Pro | Gly | Phe | Lys | Arg | Phe |
|     | 1   |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Cys | Leu | Ser | Ile | Ser | Ser | Ser | Trp | Asp | Tyr | Arg | His | Ala | Pro | Pro |
|     | 20  |     |     |     |     |     | 25  |     |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Pro | Ala | Asn | Phe | Val | Phe | Leu | Val | Glu | Thr | Gly | Phe | Leu | His | Val |
|     | 35  |     |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Xaa | Ala | Gly | Leu | Lys | Leu | Pro | Thr | Ser | Gly | Asp | Pro | Pro | Ala | Trp |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ser | Gln | Ser | Ala | Gly | Ile | Thr | Gly | Val | Xaa | Thr | Ala | Pro | Gly | Pro |
|     | 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

&lt;210&gt; 185

&lt;211&gt; 8

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 185

|     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Pro | Thr | Gly | Val | Gln | Trp | His |
|     | 1   |     |     |     | 5   |     |     |

<210> 186

<211> 8

<212> PRT

<213> Homo sapiens

<400> 186

Ala Gln Thr Gly Val Gln Trp His

1

5

1



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/14926

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.3, 69.1, 320.1, 400, 320.1; 536/ 23.1; 424/ 189.1; 530/388.1, 350; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

SEQ SEARCH SEQ ID NO: 11 and 60. EAST, Derwent  
secreted proteins**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category*           | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                           | Relevant to claim No. |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X<br>--<br>Y        | Database EST, Accession Number AW151201, NCI-CGAP<br>http://www. ncbi. nlm.nih gov/ncicgap Nation Cancer Institute,<br>Cancer Genomy Anatomy Project (CGAP), Tumor Gene Index, is<br>99% identical to SEQ ID NO: 11 from 782-1259 (see alignment),<br>1997.. | 1-10<br>---<br>11-20  |
| X<br>--<br>Y        | EP 0 490 410 A1 (SHIONOGI SEIYAKU KK) 17 June 1992,<br>Sequence Q25388: the complement is 86% identical to SEQ ID NO:<br>11 from 962-1275 (see alignment).                                                                                                   | 1-10<br>---<br>11-20  |
| X, P<br>---<br>Y, P | Database GenEMBL, Accession Number AP001456, Direct<br>Submission, Hattori, M. et al., 15 March 2000, is 98.6% identical<br>to SEQ ID NO: 11 from 7-1274.                                                                                                    | 1-10<br>---<br>11-20  |

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

|         |                                                                                                                                                                           |          |                                                                                                                                                                                                                                                       |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| *<br>** | Special categories of cited documents:<br>document defining the general state of the art which is not considered<br>to be of particular relevance                         | **<br>** | later document published after the international filing date or priority<br>date and not in conflict with the application but cited to understand<br>the principle or theory underlying the invention                                                 |
| *E*     | earlier document published on or after the international filing date                                                                                                      | *X*      | document of particular relevance, the claimed invention cannot be<br>considered novel or cannot be considered to involve an inventive step<br>when the document is taken alone                                                                        |
| *L*     | document which may throw doubts on priority claim(s) or which is<br>cited to establish the publication date of another citation or other<br>special reason (as specified) | *Y*      | document of particular relevance, the claimed invention cannot be<br>considered to involve an inventive step when the document is<br>combined with one or more other such documents, such combination<br>being obvious to a person skilled in the art |
| *O*     | document referring to an oral disclosure, use, exhibition or other<br>means                                                                                               |          |                                                                                                                                                                                                                                                       |
| *P*     | document published prior to the international filing date but later than<br>the priority date claimed                                                                     | *A*      | document member of the same patent family                                                                                                                                                                                                             |

Date of the actual completion of the international search


21 AUGUST 2000

Date of mailing of the international search report

10 OCT 2000

 Name and mailing address of the ISA/US  
 Commissioner of Patents and Trademarks  
 Box PCT  
 Washington, D.C. 20231

Facsimile No. (703) 305-3230

 Authorized officer  
  
 MARY K. ZEMAN

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/14926

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*           | Citation of document, with indication, where appropriate, of the relevant passages                                                                           | Relevant to claim No. |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X, P<br>---<br>Y, P | Database GenEMBL, Accession Number HSI1057J7, Direct Submission, PAVITT, R., is 99.6% identical to SEQ ID NO: 11 from 7-842 (see alignment), 17 August 1999. | 1-10<br>---<br>11-20  |

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/14926**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-20 in part, SEQ ID NOs 11 and 60

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/14926A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (7):

C12Q 1/68; C12N 15/09, 7/00, 5/00; C07H 21/04; C07K 17/00, 1/00, 14/00, 16/00; A61K 39/29

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL.:

435/6, 69.3, 69.1, 320.1, 400, 320.1; 536/ 23.1; 424/ 189.1; 530/388.1, 350; 514/44

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 11 and corresponding encoded polypeptide SEQ ID NO: 60, methods of making and methods of using those sequences.

Group II, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 12, corresponding encoded polypeptide SEQ ID NO: 61 and methods.

Group III, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 13, corresponding encoded polypeptide SEQ ID NO: 62 and methods.

Group IV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 14 and corresponding encoded polypeptide SEQ ID NO: 63, methods of making and methods of using those sequences.

Group V, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 15, corresponding encoded polypeptide SEQ ID NO: 64 and methods.

Group VI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 16, corresponding encoded polypeptide SEQ ID NO: 65 and methods.

Group VII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 17 and corresponding encoded polypeptide SEQ ID NO: 66, methods of making and methods of using those sequences.

Group VIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 18, corresponding encoded polypeptide SEQ ID NO: 67 and methods.

Group IX, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 19, corresponding encoded polypeptide SEQ ID NO: 68 and methods.

Group X, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 20, corresponding encoded polypeptide SEQ ID NO: 69 and related methods.

Group XI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 21 and corresponding encoded polypeptide SEQ ID NO: 70, methods of making and methods of using those sequences.

Group XII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 22, corresponding encoded polypeptide SEQ ID NO: 71 and methods.

Group XIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 23, corresponding encoded polypeptide SEQ ID NO: 72 and methods.

Group XIV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 24 and corresponding encoded polypeptide SEQ ID NO: 73, methods of making and methods of using those sequences.

Group XV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 25, corresponding encoded polypeptide SEQ ID NO: 74 and methods.

Group XVI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 26, corresponding encoded polypeptide SEQ ID NO: 75 and methods.

Group XVII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 27 and corresponding encoded polypeptide SEQ ID NO: 76, methods of making and methods of using those sequences.

Group XVIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 28, corresponding encoded polypeptide SEQ ID NO: 77 and methods.

Group XIX, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 29, corresponding encoded polypeptide SEQ ID NO: 78 and methods.

Group XX, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 30, corresponding encoded polypeptide SEQ ID NO: 79 and methods.

Group XXI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 31 and corresponding encoded polypeptide SEQ ID NO: 80, methods of making and methods of using those sequences.

Group XXII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 32, corresponding encoded polypeptide SEQ ID NO: 81 and methods.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/14926

Group XXIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 33, corresponding encoded polypeptide SEQ ID NO: 82 and methods.

Group XXIV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 34 and corresponding encoded polypeptide SEQ ID NO: 83, methods of making and methods of using those sequences.

Group XXV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 35, corresponding encoded polypeptide SEQ ID NO: 84 and methods.

Group XXVI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 36, corresponding encoded polypeptide SEQ ID NO: 85 and methods.

Group XXVII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 37 and corresponding encoded polypeptide SEQ ID NO: 86, methods of making and methods of using those sequences.

Group XXVIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 38, corresponding encoded polypeptide SEQ ID NO: 87 and methods.

Group XXIX, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 39, corresponding encoded polypeptide SEQ ID NO: 88 and methods.

Group XXX, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 40, corresponding polypeptide SEQ ID NO: 89 and related methods.

Group XXXI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 41 and corresponding encoded polypeptide SEQ ID NO: 90, methods of making and methods of using those sequences.

Group XXXII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 42, corresponding encoded polypeptide SEQ ID NO: 91 and methods.

Group XXXIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 43, corresponding encoded polypeptide SEQ ID NO: 92 and methods.

Group XXXIV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 44 and corresponding encoded polypeptide SEQ ID NO: 93, methods of making and methods of using those sequences.

Group XXXV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 45, corresponding encoded polypeptide SEQ ID NO: 94 and methods.

Group XXXVI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 46, corresponding encoded polypeptide SEQ ID NO: 95 and methods.

Group XXXVII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 47 and corresponding encoded polypeptide SEQ ID NO: 96, methods of making and methods of using those sequences.

Group XXXVIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 48, corresponding encoded polypeptide SEQ ID NO: 97 and methods.

Group XXXIX, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 49, corresponding encoded polypeptide SEQ ID NO: 98 and methods.

Group XL, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 50, corresponding encoded polypeptide SEQ ID NO: 99 and related methods.

Group XLI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 51 and corresponding encoded polypeptide SEQ ID NO: 100, methods of making and methods of using those sequences.

Group XLII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 52, corresponding encoded polypeptide SEQ ID NO: 101 and methods.

Group XLIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 53, corresponding encoded polypeptide SEQ ID NO: 102 and methods.

Group XLIV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 54 and corresponding encoded polypeptide SEQ ID NO: 103, methods of making and methods of using those sequences.

Group XLV, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 55, corresponding encoded polypeptide SEQ ID NO: 104 and methods.

Group XLVI, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 56, corresponding encoded polypeptide SEQ ID NO: 105 and methods.

Group XLVII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 57 and corresponding encoded polypeptide SEQ ID NO: 106, methods of making and methods of using those sequences.

Group XLVIII, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 58, corresponding encoded polypeptide SEQ ID NO: 107 and methods.

Group XLIX, claim(s) 1-20 in part, drawn to polynucleotide of SEQ ID NO: 59, corresponding encoded polypeptide SEQ ID NO: 108 and methods.

The inventions listed as Groups 1-49 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each separate and distinct polynucleotide is unrelated to each other polynucleotide such that there is no unifying technical feature that links all of the polynucleotide sequences together.